A Decrease in Plasma Factor Which Stimulates Prostaglandin I2 Synthesis in Patients with Acute Myocardial Infarction

Yasumi Uchida, M.D., Masatoshi Masuo, M.D., and Tsuneaki Sugimoto, M.D.

SUMMARY

PG I2 stimulating plasma factor was measured in patients with ischemic heart disease and age-matched controls. The plasma factor measured by bioassay using rat aorta was 3.2±0.6 (n=21), 2.7±0.8 (n=7), 1.6±1.5*** (n=18) and 2.2±0.06** (n=3) PG I2 ng/mg rat aorta (mean±SD; * p<0.05, ** p<0.01, *** p<0.001) in control, angina pectoris, acute myocardial infarction and old myocardial infarction with angina pectoris groups, respectively. The plasma factor measured by radioimmunoassay was 43.5±19.5 (n=11), 35.4±13.1 (n=4), 38.5±18.0 (n=9), 26.0±20.1* (n=7) and 59.5±31.2 (n=5) ng/mg rat aortic protein/15 min in control, variant angina, stable angina, acute myocardial infarction and old myocardial infarction groups, respectively. The factor in deproteinized plasma of the acute myocardial infarction group was also smaller than that of the control group. PG I2 synthesis by rat aortic ring was inhibited by mepacrine, which inhibits phospholipase A2. The plasma factor was not inactivated by heating. The results indicate that a heat-stable plasma factor which acts on phospholipase A2 or on its surrounding reaction systems is deficient in the early stage of acute myocardial infarction. A decrease in the factor may cause PG I2 deficiency, resulting in coronary thrombosis or vasospasm and consequently in acute myocardial infarction.

Additional Indexing Words:
Plasma factor    PG I2    Acute myocardial infarction    Coronary thrombosis

CORONARY thrombosis is the major precipitating factor in acute myocardial infarction. However, the mechanisms by which abrupt coronary
Thrombosis occurs within a certain group of patients is not well known. Coronary and other vessel walls generate prostaglandin I₂ (PG I₂) which inhibits platelet aggregation and causes vasodilatation.¹⁻⁴ Synthesis of PG I₂ is regulated by many plasma factors which include accelerators and inhibitors of synthesis and breakdown.⁵⁻⁹ It is well known that a certain plasma factor which stimulates PG I₂ synthesis is deficient in hemolytic uremic syndrome, resulting in microangiopathy and hemolysis.¹⁰ There is a possibility that in patients with acute myocardial infarction, PG I₂ stimulating factor is deficient, resulting in a reduction in PG I₂ synthesis leading to coronary thrombosis. Therefore, we investigated whether or not PG I₂ stimulating factor is deficient in patients with acute myocardial infarction.

SUBJECTS AND METHODS

Subjects

The patients are summarized in Table I. Patients admitted within 12 hours after the onset of the first attack of acute myocardial infarction were used. Blood was obtained before any medical treatment. The PG I₂ stimulating plasma activity in patients with acute myocardial infarction was compared with that of an age-matched control group (patients with gastrointestinal disorders or chest pain syndrome). Also, the plasma activity in patients with stable angina pectoris was measured. Patients with diabetes mellitus were excluded.

Preparation of plasma

Blood was drawn by venipuncture using plastic syringes containing heparin (final concentration 20 IU/ml). The blood was immediately refrigerated and plasma was then obtained by centrifugation at 1,000 g for 20 min at 4°C; plasma was then stored at −70°C until use. Deproteinized plasma was obtained by heating plasma at 100°C for 30 min.

Preparation of aortic rings

Male Wistar rats 10 weeks of age were decapitated and the thoracic aorta was removed and cut into 1 mm rings.

Preparation of platelet rich plasma (PRP)

PRP (10⁹/ml) was prepared by centrifugation of rabbit venous blood at 400 g for 7.5 min at room temperature.

Bioassay

Rat aortic rings were incubated with human plasma in a shaking water bath at 37°C. Twenty μl of plasma sampled at 5, 10 and 15 min after incubation were added to 250 μl of PRP and ADP (final concentration 20 μM) to observe the inhibitory effects of the incubated plasma on platelet aggrega-
tion as measured by a Sienco aggregometer. The inhibitory effects of human plasma on platelet aggregation were compared with those of synthetized PG I₂ (Ono Pharmaceutical Co), and the PG I₂ stimulating activity was expressed as PG I₂-like activity (ng/mg wet weight of rat aorta). The bioassay method employed in this study enables measurement of PG I₂ at a given incubation time, and accordingly the balance among the factors influencing PG I₂ synthesis and breakdown.

Radioimmunoassay

The aortic rings were incubated with tris-buffered saline (TBS) in a stirring bath at 37°C. PG I₂ was measured as its stable hydrolysis product (6-keto PG F₁α) at intervals of 15 min. In the preliminary experiments, synthesis of PG I₂ after 90 min incubation (after the 6th incubation period) was negligible and the amount of PG I₂ produced during the 6th incubation period was not different from that of the 7th period. Therefore, TBS was replaced by plasma or deproteinized plasma at the beginning of the 7th incubation period. The amount of PG I₂ produced during the 7th incubation period was compared between the control and patient groups.

Analysis of site(s) of action of the plasma factor

The aortic rings were incubated with plasma and indomethacin (1–100 μM), which blocks cycloxygenase,¹¹ in order to examine whether the factor acts at the cycloxygenase level or at a higher level of the arachidonic cascade. PG G₂ and H₂ rich solution was obtained by incubating rabbit PRP (10⁹/ml, 900 μl), arachidonic acid (50 μl at 10 or 50 μM) and OKY

<table>
<thead>
<tr>
<th>Table I. Subjects Used in This Study</th>
<th>n</th>
<th>male</th>
<th>female</th>
<th>age (yo; mean±SD)</th>
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</thead>
<tbody>
<tr>
<td>Bioassay</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1. Control</td>
<td>21</td>
<td>19</td>
<td>2</td>
<td>60.5±3.2</td>
</tr>
<tr>
<td>2. Acute myocardial infarction</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td>61.2±3.4</td>
</tr>
<tr>
<td>3. Angina pectoris (effort angina 6, variant angina 1)</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>59.2±4.1</td>
</tr>
<tr>
<td>4. Old myocardial infarction with angina pectoris</td>
<td>3</td>
<td>3</td>
<td></td>
<td>59.0±6.5</td>
</tr>
</tbody>
</table>

| RIA                                 |    |      |        |                  |
| 1. Control                          | 11 | 7    | 4      | 59.4±3.5         |
| 2. Acute myocardial infarction      | 7  | 5    | 2      | 59.1±3.3         |
| 3. Angina pectoris (effort angina 6, effort and rest) (angina 4, variant angina 4) | 14 | 9    | 5      | 58.7±3.0         |
| 4. Old myocardial infarction        | 5  | 4    | 1      | 60.0±4.2         |

No significant difference in age between the control and patient groups.
1580 (50 µl at 100 µM) which blocks thromboxane synthetase. Indomethacin-treated aortic rings were incubated with the supernatant of PG G₂ and H₂ rich solution (200 µl) indomethacin (50 µM) and plasma to examine whether the plasma factor acts on PG I₂ synthetase. PG I₂ stimulating plasma activity was also examined in the presence of mepacrine (0.1–1 mM) which inhibits phospholipase A₂.¹²

**Physiochemical characters of the plasma factor**

The deproteinized plasma obtained by centriflo was heated at 100°C for 30 min to determine the heat stability of the plasma factor. Bovine heparinized fresh blood was ultrafiltrated into various fractions by molecular weight to examine the molecular weight of the plasma factor. Human blood could not be used since a large amount of fresh blood was required for this analysis.

**Statistical analysis**

Statistical comparisons were made using Student’s t-test. Differences were considered significant at p<0.05.

**RESULTS**

**PG I₂ stimulating plasma activity measured by bioassay**

PG I₂-like activity increased with increasing incubation time, at least up to 15 min, in all patients. In the acute myocardial infarction (AMI) group, the activity was significantly lower than that of the control group at 5, 10 and 15 min of incubation time. No significant difference in activity was observed between the angina pectoris (AP) and the control groups (Fig. 1). Fig. 2 shows the activity in individual patients at 5 min incubation time. At this incubation time, the activity was lower not only in the AMI but also in the group of patients with old myocardial infarction complicated with AP. No activity was observed in 2 patients with AMI, however, they had no history of taking drugs which may inhibit PG I₂ synthesis.

**Plasma activity measured by RIA**

In the presence of plasma, PG I₂ (measured as 6-keto PG F₁α) synthetized by aortic rings during 15 min was 42±21 ng/mg aortic protein in the control group. This value was 120% larger than that synthetized in the presence of TBS, indicating that plasma has the ability to augment PG I₂ synthesis. Likewise, plasma had augmentatory effects on PG I₂ synthesis in all patients groups. However, the plasma activity in the AMI group was smaller than that of the control group (Fig. 3). In order to determine in what fraction of plasma the PG I₂ stimulating activity is contained, the activity in the deproteinized plasma was also measured in the same control and patient groups. The activity in deproteinized plasma was larger than that of plasma
Fig. 1 (left). PG I\(_2\) stimulating plasma activity measured by bioassay and expressed as PG I\(_2\)-like activity. AP = angina pectoris; AMI = acute myocardial infarction; OMI + AP = old myocardial infarction associated with angina pectoris. The activity was compared between control and patient groups. * p < 0.05, ** p < 0.01, *** p < 0.001.

Fig. 2 (right). Plasma activity in individual patients after 5 min of incubation.

in the control and patient groups, but the activity in AMI was smaller than that of the control (Fig. 4).

Correlation of plasma activity to blood compositions

High density lipoprotein (HDL) accelerates and low density lipoprotein (LDL) inhibits PG I\(_2\) synthesis. However, the plasma activity measured by bioassay had no correlation to HDL, LDL or the atherogenic index (total cholesterol – HDL/HDL) (Fig. 5). Albumin inhibits synthesis and breakdown of PG I\(_2\), however, the plasma activity had no correlation to albumin concentration. Lowering pH accelerates and elevating pH inhibits breakdown of PG I\(_2\), however, the activity had no correlation to plasma pH.

Time-course changes in plasma activity in AMI

The time course changes in plasma activity were examined in 3 patients with AMI who had received no medication before blood sampling which might have affected the arachidonic cascade. The activity showed a tendency to increase in the second day in 1 but not in the other 2 patients.
Site(s) of action of the plasma factor

One $\mu$M or more of indomethacin, which inhibits cycloxygenase, reduced or eliminated PG $I_2$ synthesis by the aortic rings (Fig. 6) in the presence or absence of the plasma, indicating that the site of action of the plasma is above
Fig. 6. Effects of indomethacin on plasma activity measured by RIA. Plasma was added at the beginning of the 7th and 8th incubation periods.

Fig. 7. Effects of TBS, plasma and deproteinized plasma on PG \( \text{I}_2 \) synthesis from PG \( \text{H}_2 \). Left: 10 \( \mu \text{M} \) arachidonic acid was added. Right: 50 \( \mu \text{M} \) arachidonic acid was added.

the cyclooxygenase level of the arachidonic cascade. The plasma factor had no stimulating action on PG \( \text{I}_2 \) from PG \( \text{H}_2 \) (Fig. 7). In the presence of mepacrine, which inhibits phospholipase \( \text{A}_2 \), the aortic rings could not synthetize PG \( \text{I}_2 \) and the plasma could not antagonize the action of mepacrine (Fig. 8). Ca\(^{2+}\) which is required for phospholipase \( \text{A}_2 \) activity, could not stimulate
Fig. 8. Effects of mepacrine on plasma activity. Plasma was added at the beginning of the 7th and 8th incubation periods.

Fig. 9. PG I₂ stimulating activity in fractions of various molecular weight. MW=molecular weight; YM and YC=filters used for ultrafiltration. Each fraction was added at the beginning of the 7th and 8th incubation periods.

PG I₂ synthesis.

Physiochemical properties of the plasma factor

Deproteinized plasma obtained by centriflo was heated at 100°C for 30 min; however, the plasma activity was not altered. The bovine deproteinized plasma was ultrafiltrated into various molecular weight fractions, and
the activity in each fraction was examined. The PG \( I_2 \) stimulating activity was observed in the fractions of below 5,000, 1,000 and even in the fraction of below 300 Daltons (Fig. 9).

**Discussion**

The results of this study indicate that a heat stable PG \( I_2 \) stimulating factor is deficient in the early stage of acute myocardial infarction. There are a number of factors in plasma which affect PG \( I_2 \) synthesis and breakdown. McIntyer factor is heat-unstable \(^6\) and its deficiency in hemolytic uremic syndrome \(^{10}\) and thrombolic thrombocytopenic purpura has been demonstrated. Heat-stable factors were also found by Remuzzi and Ritter. \(^6\), \(^{10}\) In addition to these factors whose chemical structures are not known, estradiol, \(^{12}\) vitamins \( C \), \( E \), \(^{16}\) insulin, \(^{17}\) HDL, \(^{14}\) thrombin, \(^7\) and platelet derived factor \(^{18}\) stimulate PG \( I_2 \) synthesis. On the other hand, LDL, \(^{14}\) superoxides \(^8\) or \( \beta \)-thromboglobulin \(^{19}\) inhibit PG \( I_2 \) synthesis. It is generally considered that in addition to the factors that influence breakdown, the balance between the stimulating and inhibiting factors determine the degree of PG \( I_2 \) synthesis by vessel walls and the local or systemic PG \( I_2 \) concentration. In this study, the plasma activity measured by bioassay had no correlation to the atherogenic index, albumin concentration or pH, indicating that the factor examined in this study was not LDL, albumin or pH. The factor found in this study was heat-stable as were the factors described by Remuzzi and Ritter, and therefore different from the McIntyer factor. However, we did not determine whether the factor in this study was the same as that of Remuzzi or Ritter.

The factor in bovine plasma was found not only in the high molecular weight fractions but also in the fraction of below 300 Daltons. This indicates that, at least in bovine plasma, a part of the factor is of low molecular weight. Since the weight of human plasma factor was not examined, it is not known whether the human plasma factor is the same as the bovine factor.

PG \( I_2 \) synthesis from PG \( H_2 \) was not accelerated by the plasma factor, while the action of the plasma factor was blocked by mepacrine which inhibits phospholipase \( A_2 \). \(^{19}\) Although there is a possibility that mepacrine in high concentrations may have inhibited nonspecifically the synthesis of PG \( I_2 \), this fact may suggest that the plasma factor acted not on PG \( I_2 \) synthetase but on phospholipase \( A_2 \) or on its surrounding reaction systems.

Although whether or not the plasma factor decreases preceding the attack was not determined, there is a possibility that PG \( I_2 \) stimulating plasma factor decreased, leading to a decrease in PG \( I_2 \) production causing coronary thrombosis and/or vasospasm, resulting in acute myocardial infarction.
REFERENCES


