Preexisting Antibodies to Platelet Factor 4-Heparin Complexes in Patients with Acute Coronary Syndrome Who Have No History of Heparin Exposure

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Key Words
Heparin-induced thrombocytopenia · Heparin-platelet factor 4 complex antibodies · Acute coronary syndrome · Thrombotic complications

Abstract
Preexisting heparin-induced thrombocytopenia (HIT) antibodies are detected in some patients who have not previously been exposed to any kind of heparin. However, the role of preexisting HIT antibodies in acute coronary syndrome (ACS) is still unknown. This study was carried out to clarify the role of preexisting HIT antibodies in patients with ACS. Forty patients with ACS who had not been exposed to any kind of heparin via the venous or subcutaneous route or heparin-coated materials and had undergone percutaneous coronary intervention (PCI) under heparin anticoagulation were chosen from the medical records in the cardiology emergency department. As a control of the ACS patients, 51 patients with angina pectoris who underwent elective PCI under heparin anticoagulation were chosen in the same manner as the ACS patients. Preexisting HIT antibodies were detected by ELISA in 6 patients. Two of the 6 patients developed HIT and 1 patient experienced thrombosis requiring intracoronary thrombolytic therapy. Thrombotic complications during and immediately after PCI in the very early stage after heparin administration were found in 4 of 6 patients with preexisting HIT antibodies. The frequency of preexisting HIT antibodies in ACS patients was significantly increased in comparison with that in non-ACS patients. The odds ratio of the risk of thrombotic complication between ACS and non-ACS patients was estimated at 8.82 (95% CI: 1.3–63). Also, preexisting HIT antibodies in ACS patients significantly increased the risk of thrombotic complications compared with ACS without preexisting HIT antibodies. In conclusion, ACS patients with positive HIT antibodies have an increased risk of thrombotic complications during PCI performed under anticoagulation with heparin.

Introduction
Heparin is routinely administered to patients with acute coronary syndrome (ACS) as an anticoagulant during cardiac intervention. Heparin-induced thrombocytopenia (HIT) has been reported to occur less frequently after an initial heparin administration, due to an immunological mechanism involving the formation of complexes between exogenous heparin from animal sources and antibodies to platelet factor 4 (PF4)-heparin complexes. HIT can often lead to arterial, venous or micro-
vascular thrombosis. Thrombocytopenia in HIT is mainly mediated by immune complexes comprising antibodies to PF4-heparin complex and PF4-heparin complex as antigen in circulation. The immune complex can bind to FcγRIIA receptors on the surface of platelets, and activated platelets release procoagulant materials into the circulation. Finally, HIT sets up a hypercoagulability.

Although the onset of classical HIT has been reported to be 5–14 days after heparin anticoagulation, HIT antibodies were detected in cardiac patients who had not been exposed to heparin using an immune antigen assay [1]. The frequency of preexisting HIT antibodies is estimated at 1.4% in normal, 4.5% in elderly, 4.8% in pregnant and 8.6% in diabetic subjects [2]. To explain the preexistence of HIT antibodies, it was proposed that heparin-like molecules (glycosaminoglycans) on injured endothelium bind with PF4 to form complexes with which the antibodies react. Neoantigens appeared to cause conformational change of PF4 to induce the production of heterogenetic antibodies in the Ig subclass in the presence of heparin-like molecules. In ACS, a high level of PF4 associated with the activated platelets facilitates production of heparin on therapeutic heparin infusion [3]. HIT would be likely to develop in ACS patients with preexisting HIT antibodies. Also, HIT is known to be complicated by thrombosis in the presence of HIT antibodies. This study was carried out to clarify whether preexisting HIT antibodies can be used to predict the risk of thrombotic complications during or after cardiac intervention in ACS.

Materials and Methods

Forty consecutive patients with ACS were chosen based on the following criteria: no exposure to heparin confirmed in their medical records and the performance of percutaneous coronary intervention (PCI) within 6 h of the onset of ACS. ACS was diagnosed based on clinical symptoms, electrocardiographic ST change with or without Q waves, creatine kinase MB isozyme elevation, and left ventricular dysfunction of the infarct area as evaluated using left ventricular angiography and echocardiograms. Among non-ACS patients with no history of heparin exposure confirmed in their medical records, 51 consecutive patients with a stable angina pectoris were selected as controls from medical records covering the same period as the survey of ACS patients. The diagnosis of non-ACS patients was based on clinical symptoms and an exercise tolerance test. In all subjects, culprit lesions of the coronary artery were clearly demonstrated by coronary angiography. The two groups showed no differences in age, gender, and risk factors such as frequency of hypertension, diabetes mellitus, cholesterol level, high-density lipoprotein concentrations, smoking habits or BMI (table 1). High levels of triglyceride were found in non-ACS patients. Follow-up platelet counts were routinely done before, at the end of, and 5 days after heparin treatment. Frozen serum samples obtained before heparin treatment were examined for heparin-PF4 complex antibodies (HIT antibodies). The measurement of HIT antibodies was carried out with an ELISA (Asserachrom HPIA, Stago, France). Using this assay, the optical density at 492 nm for HIT antibodies in the Ig subclass in the presence of heparin-like molecules (glycosaminoglycans) on injured endothelium bind with PF4 to form complexes with which the antibodies react. Neoantigens appeared to cause conformational change of PF4 to induce the production of heterogenetic antibodies in the Ig subclass in the presence of heparin-like molecules. In ACS, a high level of PF4 associated with the activated platelets facilitates production of heparin on therapeutic heparin infusion [3]. HIT would be likely to develop in ACS patients with preexisting HIT antibodies. Also, HIT is known to be complicated by thrombosis in the presence of HIT antibodies. This study was carried out to clarify whether preexisting HIT antibodies can be used to predict the risk of thrombotic complications during or after cardiac intervention in ACS.

Table 1. Characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ACS (n = 40)</th>
<th>Non-ACS (n = 51)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, males/females</td>
<td>29/11</td>
<td>36/15</td>
<td>0.84</td>
</tr>
<tr>
<td>Age, years</td>
<td>64.4 ± 2.0</td>
<td>64.6 ± 1.6</td>
<td>0.84</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.1 ± 0.7</td>
<td>24.5 ± 0.4</td>
<td>0.63</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28 (70)</td>
<td>27 (53)</td>
<td>0.10</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>199 ± 6.4</td>
<td>211 ± 6.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>96 ± 9.4</td>
<td>156 ± 9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-density lipoprotein, mg/dl</td>
<td>40 ± 1.5</td>
<td>45 ± 3.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>17 (43)</td>
<td>13 (25)</td>
<td>0.09</td>
</tr>
<tr>
<td>Active smokers</td>
<td>28 (70)</td>
<td>27 (53)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Figures in parentheses represent percentage.
Results

HIT antibodies before heparin infusion, preexisting HIT antibodies, were found in 6 of 40 ACS patients who had never been exposed to heparin and in 1 of the non-ACS patients. Preexisting HIT antibodies were more common in ACS patients (p = 0.03). Two patients developed thrombocytopenia 9 and 8 h after the administration of heparin, respectively. These 2 patients were diagnosed with HIT, 1 patient having >50% thrombocytopenia with no other cause, and the other patient >30% thrombocytopenia complicated by thrombotic occlusion during PCI (table 2). Three of 4 positive patients without thrombocytopenia showed thrombotic complications during and after PCI. Although there were no significant differences in mean age or other risk factors mentioned in table 1 between ACS patients with and without preexisting HIT antibodies, significant differences were found in both groups and included a more prominent reduction in the platelet count (p = 0.02) and more frequent formation of a thrombus (odds ratio 8.2, CI: 1.4–63.2, p = 0.026) (table 3). In non-ACS patients, no HIT was found and 1 patient without preexisting HIT antibodies developed a thrombus during PCI. Thrombotic complications requiring thrombolytic therapy were found in 10 patients of the ACS group and 1 patient of the non-ACS group. The frequency of thrombotic complications was significantly higher in the ACS than the non-ACS group (p = 0.003).

Platelet counts in ACS patients were significantly reduced on cessation of heparin treatment and returned to preheparin levels. No significant reduction in the platelet count was induced by the administration of heparin in non-ACS patients (fig. 1).

Optical densities obtained from ELISA in both groups showed significantly higher titers in ACS than non-ACS (p < 0.01) (fig. 2). The level of HIT antibodies was higher in ACS with negative titers than in non-ACS with negative titers (p < 0.05). ACS patients had a much greater chance of generating HIT antibodies than non-ACS patients. Preexisting HIT antibodies detected by ELISA appeared more frequently in ACS patients and facilitated thrombotic complications during and immediately after PCI under anticoagulation with heparin (table 3).

<table>
<thead>
<tr>
<th>No.</th>
<th>Clinical diagnosis</th>
<th>Age (sex)</th>
<th>Main coronary artery lesion</th>
<th>Thrombotic occlusiona</th>
<th>Heparin administration, h</th>
<th>Reduction of platelet count at heparin cessation, %</th>
<th>Preexisting HIT antibodies</th>
<th>ELISA</th>
<th>HIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACS</td>
<td>67 (M)</td>
<td>CX</td>
<td>–</td>
<td>12</td>
<td>19.4</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ACS</td>
<td>59 (M)</td>
<td>RCA</td>
<td>+</td>
<td>1</td>
<td>20.3</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>ACS</td>
<td>57 (M)</td>
<td>LAD</td>
<td>–</td>
<td>9</td>
<td>61.8</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td>ACS</td>
<td>75 (M)</td>
<td>RCA</td>
<td>+</td>
<td>8</td>
<td>42.5</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ACS</td>
<td>63 (M)</td>
<td>RCA</td>
<td>+</td>
<td>6</td>
<td>23.2</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ACS</td>
<td>69 (M)</td>
<td>LAD</td>
<td>+</td>
<td>9</td>
<td>16.2</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>non-ACS</td>
<td>62 (F)</td>
<td>LAD</td>
<td>–</td>
<td>48</td>
<td>21.7</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

HIPA = Heparin-induced platelet aggregation; CX = left circumflex coronary artery; RCA = right coronary artery; LAD = left anterior descending artery.

a Failure of repeated angioplasty and required thrombolytic therapy.
b HIT.
c HIT with thrombosis.

Table 2. Clinical characteristics of 7 patients with preexisting HIT antibodies

<table>
<thead>
<tr>
<th>Preexisting HIT antibodies</th>
<th>Thrombocytopenia (&gt;30% fall) at end of heparin</th>
<th>Thrombotic complications</th>
<th>Odds ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (n = 6)</td>
<td>2</td>
<td>4</td>
<td>9.3 (1.4–63.2)</td>
<td>p = 0.026</td>
</tr>
<tr>
<td>Negative (n = 34)</td>
<td>0</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Some studies have provided evidence that endothelial damage contributes to thrombotic complications in HIT. Serum from HIT patients reacted with the damaged endothelium and affected the expression of tissue factor, caused a fibrinolytic deficit, and increased levels of soluble adhesion molecules [6]. The target of HIT antibodies was not only PF4 bound to heparin, but also PF4 in complexes with heparin-like molecules on the remodeled endothelium [7]. Also, the tissue factor expression plays an important role in thrombogenicity in the coronary atherosclerotic plaques in ACS patients [8]. In this study, ACS patients with preexisting HIT antibodies detected by ELISA frequently had thrombotic complications during PCI under anticoagulation with heparin. Thrombotic complications requiring intracoronary thrombolytic treatment were suspected to be more common in ACS patients carrying HIT antibodies.

A rapid onset type of HIT occurred very shortly after heparin infusion in patients who possessed HIT antibodies although the same patients showed no thrombocytopenia on recent heparin exposure. This type of HIT is defined by an unexpected fall in the platelet count caused by residual circulating HIT antibodies [9]. HIT antibodies are thought to be a novel, independent predictor of myocardial infarction at 30 days in patients presenting with ACS [10]. In this study, subjects with no history of heparin exposure were selected from a thorough survey of medical records. Two HIT patients were found among 40 ACS patients suffering from thrombocytopenia and thrombotic complications during PCI. It was impossible to suspect that these patients with preexisting HIT antibodies were developing HIT shortly after heparin treatment. Two of the other 4 ACS patients with preexisting HIT antibodies developed thrombotic complications during PCI. Thus, cardiac patients with positive HIT antibodies who underwent CABG should not undergo surgery until the confirmation of negative seroconversion and avoid the use of heparin before and after surgery [11].

HIT antibodies detected in both ELISA and platelet aggregation assays were found in 11 out of 757 inpatients with no heparin exposure, suggesting that PF4 released from activated platelets could bind to endogenous heparin-like molecules on the endothelium and induce conformational changes of PF4 to neoantigen [2]. Early-onset HIT (<5 days) was found in 3 of 76 serologically confirmed HIT patients with initially exposed heparin. As long as no HIT antibodies remain in the circulation, it is thought to be unlikely that HIT occurs within the first days of heparin treatment [12].

Based on the experience of this study, it is suggested that the preexisting HIT antibodies in patients with no history of heparin exposure were likely to predispose these individuals to early-onset HIT. Also, ACS patients who possess preexisting HIT antibodies developed throm-

Fig. 1. Platelet count before and immediately after heparin treatment in ACS and non-ACS patients (mean ± SD).

Fig. 2. HIT antibody titers of ELISA in three groups.
botic complications during PCI under initial heparin exposure even if they had no thrombocytopenia. Indeed, ACS patients compared with non-ACS patients are prone to enter a hypercoagulability [13], and the manipulation of PCI itself induces thrombogenicity due to artificial endothelial damage [14]. ACS patients with preexisting HIT antibodies showed more hypercoagulability on the addition of exogenous heparin, because they already had HIT antibodies together with both activated platelets and a damaged endothelium. Although this study examined only a relatively small number of ACS patients and patients with preexisting HIT antibodies, the following conclusion can be made. In order to prevent the development of HIT and thrombotic complications in ACS patients with preexisting HIT antibodies, it is necessary to recognize early-onset HIT in ACS patients without any history of heparin exposure and to treat these patients with alternatives to heparin.

References

[2] Leitz H, Walenga JM, Fabbri N, Lewis BE, Fasanella A, Messmore HL, Pifarre R: Pre-existence of anti-PF4-heparin antibodies in pa-
[10] Williams RT, Damara-
ju LV, Mascelli MA, Barnathan ES, Califf RM, Simoons ML, De-
largyris EN, Sane DC: Anti-platelet factor 4/ heparin antibodies, an independent predictor of 30-day myocardial infarction after acute 
thrombocytopenia, temporal pattern of thrombocytopenia in relation to initial use or reexpo-
adjunctive anticoagulant (argatroban vs. hepa-
rin) during and after elective percutaneous transluminal coronary angioplasty on inflam-