Role of 4 platelet membrane glycoprotein polymorphisms on experimental arterial thrombus formation in men

Yves Cadroy, Kjell S. Sakariassen, Jean-Paul Charlet, Claire Thalamas, Bernard Boneu, and Pierre Sie

This study investigates whether the polymorphisms of 3 important platelet receptors affected experimental thrombus formation in men. Forty healthy male volunteers randomly recruited were genotyped for the variable number of tandem repeat (VNTR) of GPIbα, the −5T/C polymorphism in the Kozak sequence of GPIIbα, the 807C/T polymorphism of GPIa, and the PlA1/PlA2 polymorphism of GPIIb-IIIa. Platelet thrombus formation was induced ex vivo by exposing a collagen-coated coverslip in a parallel plate perfusion chamber to native blood for 4 minutes. The shear rates at the collagen surface were 650 and 2600 s⁻¹. At 2600 s⁻¹ platelet thrombus formation was significantly related only to the 807C/T polymorphism. In contrast, at 650 s⁻¹ thrombus formation was significantly altered only by the Kozak sequence polymorphism. The VNTR and the PlA1/PlA2 polymorphisms did not influence thrombus formation. Thus, platelet thrombus formation is significantly influenced by genetic variations of the GPIbα and GPIa receptors. The effect of these polymorphisms was dependent on the blood flow rate. (Blood. 2001;98:3159-3161)

Brief report

Introduction

Arterial thrombosis is a process that involves platelet adhesion and aggregation. These events are mediated through the interactions of platelet membrane glycoproteins (GPs) with adhesive substrates. Platelet adhesion to collagen is one of the first steps of thrombus formation. von Willebrand factor mediates an initial tethering of platelets to collagen via GPIbα. Platelets translocate along the collagen surface until movement is arrested by firm engagement of the platelet membrane collagen receptor GPIa-IIa (integrin α2β1). As a consequence of platelet activation and inside-out signaling, GPIIb-IIIa (integrin α5β3) acquires the ability to bind fluid phase fibrinogen or von Willebrand factor and thereby trigger platelet aggregation.

The 3 major platelet membrane–adhesive receptors GPIbα, GPIa-IIa, and GPIIb-IIIa present genetic polymorphisms. A number of clinical studies have reported conflicting data on the association of these polymorphisms and arterial thrombosis. However, these studies were often too small and heterogeneous with regard to the type of patients involved, the pathology, and the treatment. Information directly regarding the effect of these genetic changes on experimental thrombosis is lacking. In addition, no study has compared the respective role of these important genetic factors thought to be associated with an increased tendency to thrombosis.

The aim of the present study was to assess the role of 4 polymorphisms of these 3 platelet receptors that potentially are linked to an increased thrombotic tendency. We used an experimental model of arterial thrombus formation device in men that closely mimics relevant clinical situations. In this model, native blood is drawn from healthy volunteers through a parallel-plate chamber device where blood components interact with collagen at well-established flow conditions. Blood flow conditions mimic wall shear rates as encountered in medium-sized (650 s⁻¹) and moderately stenosed small (2600 s⁻¹) arteries. This model has been used to investigate numerous antithrombotic strategies, and the results appear consistent with clinical data. Four polymorphisms were investigated. They were the variable number of tandem repeats (VNTRs) of GPIbα, the −5T/C polymorphism in the Kozak sequence of GPIIbα, the 807C/T polymorphism of GPIa, and the PlA1/PlA2 polymorphism of GPIIb-IIIa.

Study design

Subjects

The study population consisted of 40 healthy Caucasian male volunteers aged 21 to 41 years (mean ± 1 SD, 25 ± 4). They had no history or clinical signs of any disease. Clinical chemistry, hematologic, and hemostatic laboratory values were within the normal ranges. All subjects gave written, informed consent to the protocol, which was approved by the local Human Subjects Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Toulouse).

The volunteers were requested to come to the study center between 9:00 and 10:00 AM after 12 hours of fasting. They were not taking any medication known to affect blood coagulation or platelet function during the 10 days preceding the blood donations. In addition, all volunteers were nonsmoking subjects or smoking less than 10 cigarettes/day, and they did not smoke on the day of the perfusion experiments. We have

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previously shown that smoking does not affect platelet thrombus formation in these conditions. Their body mass index was less than 25 kg/m².

Genotyping of the 4 polymorphisms

Genomic DNA was isolated from peripheral blood mononuclear cells, and the polymorphisms were determined by genomic polymerase chain reaction according to methods previously described.8–11

Preparation of thrombogenic surface

Human type I collagen purified from pepsin-digested placenta collagens (Sigma, Saint-Quentin-Fallavier, France) was used. A fibrillar collagen suspension was obtained by dialysis at 4°C against 20 mM NaHPO₄, pH 7.5, for 24 hours. It was spray-coated onto Thermaxan plastic coverslips (Miles Laboratories, Naperville, IL) to a final density of 5 µg/cm². The coverslips were stored at room temperature for 15 to 20 hours before use.³

Perfusion experiments

Perfusion experiments were performed with a parallel-plate perfusion chamber device at 37°C.³,4 Following blood sample collection, native blood was drawn directly from an antecubital vein of the volunteers by a 19-gauge infusion set (Ohmeda, Helsingborg, Sweden) over the collagen-coated coverslip positioned in the parallel-plate perfusion chamber. The blood flow rate was maintained at 10 mL/minute by a peristaltic roller pump (Minipuls, Gilson, Villiers-Le-Bel, France) placed distal to the chamber. The wall shear rates were 650 and 2600 s⁻¹. The blood perfusion experiment lasted for 4 minutes and was followed by a 30-second perfusion of phosphate-buffered saline at the same flow rate to wash out blood from the flow channel of the perfusion chamber. Subsequently, the coverslip covered by thrombotic deposits was placed in a plasmin solution and processed as previously described.⁵

Immunologic determination of platelet deposition

Platelet deposition was quantified by measurement of a specific platelet α granule membrane protein, P-selectin.¹³ After centrifugation of the plasmin-digested thrombus, the pellet was dissolved in 400 µL lytic buffer, frozen and thawed 3 times, and then sonicated (4°C, 20 KHz) for 270 seconds. All samples of dissolved pellets were stored at −80°C until assayed for P-selectin by immunoenzymoassay (Bender MedSystems, Vienna, Austria). Results were expressed as the number of platelets deposited/cm² (×10¹⁰/cm²).

Other laboratory procedures

von Willebrand factor plasma levels were measured immunologically, using the Laurell method (Assera-vWF; Stago). Fibrinogen plasma levels were measured by the von Clauss method on an STA automate (Stago, Asnières, France). Hematocrit, white blood cell, and platelet counts were measured by an electronic counting device (Model S plus; Coulter Electronics, Hialeah, FL).

Statistical analysis

Results were expressed as the mean ± 1 SD. The relationship between the 4 polymorphisms and platelet deposition was analyzed by using a stepwise multiple linear regression analysis. This relationship was determined by adjusting other important blood parameters susceptible to influence platelet thrombus formation (ie, individual platelet count, white blood cell count, hematocrit, fibrinogen, and von Willebrand factor plasma levels). Two group comparisons were performed by using the nonparametric Mann-Whitney U test. All statistical tests of hypothesis were 2-tailed and were performed at .05 level of significance.

Results and discussion

The characteristics of platelet thrombus formation in blood from volunteers with the 4 polymorphisms are shown in Table 1.

<table>
<thead>
<tr>
<th>Shear rate (s⁻¹)</th>
<th>n</th>
<th>Platelet deposition (× 10¹⁰/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>650</td>
<td>2600</td>
</tr>
<tr>
<td>GP Ib Kozak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>32</td>
<td>0.85 ± 0.42</td>
</tr>
<tr>
<td>C/T</td>
<td>8</td>
<td>1.38 ± 0.60</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>GP Ib VNTR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/B</td>
<td>1</td>
<td>1.14</td>
</tr>
<tr>
<td>B/C</td>
<td>7</td>
<td>1.00 ± 0.34</td>
</tr>
<tr>
<td>C/C</td>
<td>24</td>
<td>0.96 ± 0.51</td>
</tr>
<tr>
<td>C/D</td>
<td>8</td>
<td>0.87 ± 0.64</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>GPIa 807</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>17</td>
<td>0.94 ± 0.45</td>
</tr>
<tr>
<td>C/T</td>
<td>17</td>
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</tr>
<tr>
<td>T/T</td>
<td>6</td>
<td>1.07 ± 0.91</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>GPIb/IIa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1/H²A¹A¹</td>
<td>21</td>
<td>0.97 ± 0.42</td>
</tr>
<tr>
<td>P1/H²A¹A¹</td>
<td>19</td>
<td>0.94 ± 0.58</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

The relationship between the 4 polymorphisms and platelet deposition was analyzed by using a stepwise multiple linear regression analysis. This relationship was determined by adjusting other important blood parameters susceptible to influence platelet thrombus formation (ie, individual platelet count, white blood cell count, hematocrit, fibrinogen, and von Willebrand factor plasma levels). GP, glycoprotein; VNTR, variable number of tandem repeat; NS = not significant. Mean ± SD.
note that the 807C/T and Kozak polymorphisms were not linked (NS, Fisher test) and that there was no interaction between these 2 polymorphisms.

In conclusion, our results support the clinical studies reporting a positive association between the Kozak and 807C/T polymorphisms and acute arterial thrombotic events. In addition, our studies may explain at least some of the controversies reported between these genetic variations and the thrombotic phenotype, because the effect of this polymorphism on platelet thrombus formation depends on the local blood flow conditions. The potential effect of this polymorphism on arterial thrombogenesis must be given consideration, because fibrillar collagens are major components of the subendothelial matrix that trigger thrombus formation.

References

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