Human Type II Hyperlipoproteinemia Enhances Platelet-Collagen Adhesion in Flowing Nonanticoagulated Blood

Yves Cadroy, Sylvie Lemozy, Armelle Diquelou, Jean Ferrieres, Philippe Douste-Blazy, Bernard Boneu, Kjell S. Sakariassen

We investigated the effects of high plasma lipid levels on platelet adhesion and platelet thrombus formation in nonanticoagulated human blood on collagen fibrils at an arterial wall shear rate of 2600 seconds\(^{-1}\). Nonanticoagulated blood was drawn directly at a flow rate of 10 mL/min for 3 minutes from an antecubital vein of patients with type Ila (n=5) and type IIb (n=4) hyperlipoproteinemia over purified human type III collagen fibrils that were positioned on a plastic coverslip in a parallel-plate perfusion chamber. Results were compared with those obtained in healthy individuals with normal lipid plasma levels (n=9). Blood-collagen interactions were quantified by morphometry as platelet-collagen adhesion, thrombus volume, and fibrin deposition. Platelet-collagen adhesion in the two groups of patients was significantly higher than in healthy individuals (70.7 [61.2 to 82.0] and 70.3 [66.4 to 81.0] in types Ila and IIb patients, respectively, versus 51.2 [44.5 to 68.6] in control subjects; P<.05. All values are percent median [range]). In contrast, the thrombus volume was similar in the three groups (11.3 [8.0 to 13.0], 9.6 [6.4 to 15.3], and 10.2 [6.8 to 16.1] \(\mu\text{m}^3/\mu\text{m}^2\) [range], respectively). Differences in fibrin deposition were not observed. Thus, it appears that platelet-collagen adhesion is augmented in patients with type Ila and IIb hyperlipoproteinemia, indicating that the process of thrombogenesis is hastened in these patients.

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KEY WORDS • hyperlipoproteinemia • platelet adhesion • thrombus formation • arterial flow • collagen • atherosclerosis

Many studies indicate that hyperlipoproteinemia, which is a major risk factor of atherosclerosis and thrombosis, influences platelet function.\(^1\)\(^-\)\(^3\) Hyperlipoproteinemia appears to be associated with an enhancement of platelet activity, as illustrated by the increased sensitivity of such platelets to aggregating agents.\(^1\)\(^-\)\(^3\) However, the clinical relevance of these findings is questionable, since platelet aggregation tests are performed with anticoagulated platelet-rich plasma, and thus without the presence of blood flow and coagulation.

A recent study\(^4\) evaluates the consequences of very high plasma lipid levels on platelet adhesion and thrombus formation on de-endothelialized abdominal rabbit aortas in perfusion chambers at controlled blood flow conditions and shows that hyperlipoproteinemia enhances thrombus formation. This finding led us to investigate whether this was also the case in humans with well-characterized type Ila and type IIb hyperlipoproteinemias. We used an ex vivo model in which nonanticoagulated blood from patients and healthy individuals was perfused over type III collagen fibrils that were positioned in parallel-plate perfusion chambers.\(^5\) Platelet adhesion, thrombus formation, and fibrin deposition were studied at an arterial wall shear rate of 2600 seconds\(^{-1}\). Such a shear rate may be encountered at the apex of moderately stenosed coronary arteries.

**Methods**

**Subjects**

Five patients classified as having type Ila hyperlipoproteinemia and 4 patients with type IIb hyperlipoproteinemia according to World Health Organization criteria\(^6\) were recruited. They were compared with 9 healthy volunteers from the clinical staff of our hospital. The main clinical characteristics of these subjects are presented in Table 1. They had no history of thromboembolic disorders. None had ingested any lipid-lowering medications for at least 1 month before the perfusion procedure. All subjects stated they had not taken aspirin or other drugs known to affect platelet function in the last 8 days preceding the blood donation. None of the women were on oral contraceptive drug treatment. An oral informed consent was obtained from all subjects.

**Blood Samples**

Blood samples and perfusions were performed between 9 and 10 AM after overnight fasting. Blood was collected from an antecubital vein by using a 19-gauge butterfly (SFL, Abbott, France) and was placed in tubes for serum separation for lipid measurement (SST tubes, Ref 606510, Becton Dickinson); in EDTA (Ref 606651,
TABLE 1. Clinical Characteristics of Healthy Individuals and Hyperlipoproteinemic Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy Individuals</th>
<th>Type IIA</th>
<th>Type IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Age, y</td>
<td>(25-65)</td>
<td>(35-65)</td>
<td>(51-59)</td>
</tr>
<tr>
<td>Sex ratio, M/F</td>
<td>6/3</td>
<td>3/2</td>
<td>3/1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.8</td>
<td>24.9</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>(18.9-26.2)</td>
<td>(18.6-26.9)</td>
<td>(21.5-24.2)</td>
</tr>
<tr>
<td>Duration of hyperlipidemia, y</td>
<td>20.0</td>
<td>11.5</td>
<td>(2-30) (4-20)</td>
</tr>
</tbody>
</table>

M indicates male and F, female. Values are expressed as median (range).

Morphometric Evaluation

Evaluation of thrombotic deposits was performed on Epon-embedded semithin sections (1 μm thick) stained with toluidine blue and basic fuchsin. The sections were prepared as previously described at an axial position 1 mm downstream from the flow inlet at the collagen and perpendicular to the direction of blood flow. The morphometric analysis was according to Baumgartner and Muggli and Sakariassen et al. Platelet adhesion and fibrin deposition were quantified by light microscopy as the percentage of the total evaluated surface covered with adherent platelets (contact and spread platelets) and with fibrin, respectively. Sectional thrombus area was quantified by computer-assisted morphometry and was expressed as thrombus volume (micrometers cubed) per unit surface area (micrometers squared).

Statistical Analysis

Statistical measurements were made using the PCSM program (Deltasoft, France). Results were expressed as median and range, and populations were compared using the Mann-Whitney U test. To assess the relations of platelet adhesion with the different lipid parameters, a multiple regression analysis with calculation of the standardized partial regression coefficient (β) was performed. Differences of P<.05 were considered as significant.

Results

The main biological characteristics of patients and healthy individuals are given in Table 2. Patients were classified as having type IIA or type IIB hyperlipoproteinemia according to the criteria of the World Health Organization. Type IIA hypercholesterolemic patients had high plasma levels of total and LDL cholesterol (LDL-C) (P<.01 versus the group of healthy individuals), whereas HDL-C, VLDL cholesterol (VLDL-C), and triglycerides were normal. Type IIB hyperlipoproteinemic patients had significantly increased plasma levels of triglycerides, total cholesterol, LDL-C, and VLDL-C (P<.05). Individual plasma levels of the respective lipid parameters of the group of healthy subjects were all within the normal ranges. Also, there was no difference in hematocrit, leucocyte and platelet count, plasma fibrinogen, and von Willebrand factor levels between the patient groups and the group of healthy individuals (P>.10).
TABLE 2. Biological Characteristics of Healthy Individuals and Hyperlipoproteinemic Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy Individuals</th>
<th>Type IIa</th>
<th>Type IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>42.2 (40.0-47.8)</td>
<td>41.9 (36.5-43.2)</td>
<td>48.0 (36.5-50.3)</td>
</tr>
<tr>
<td>Leucocytes, ×10^9/L</td>
<td>5.5 (4.8-11.7)</td>
<td>9.2 (6.3-11.2)</td>
<td>7.7 (4.7-13.6)</td>
</tr>
<tr>
<td>Platelets, ×10^9/L</td>
<td>279 (126-367)</td>
<td>302 (182-442)</td>
<td>266 (241-314)</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.6 (1.9-3.8)</td>
<td>3.1 (2.2-3.5)</td>
<td>2.3 (2.0-3.5)</td>
</tr>
<tr>
<td>von Willebrand factor, %</td>
<td>144 (128-285)</td>
<td>170 (93-260)</td>
<td>180 (70-200)</td>
</tr>
<tr>
<td>Total cholesterol, g/L</td>
<td>1.9 (1.5-2.3)</td>
<td>3.5 (3.3-4.5)</td>
<td>2.9 (2.5-3.3)*</td>
</tr>
<tr>
<td>HDL-C, g/L</td>
<td>0.7 (0.5-0.9)</td>
<td>0.7 (0.5-0.6)</td>
<td>0.4 (0.3-0.6)</td>
</tr>
<tr>
<td>LDL-C, g/L</td>
<td>1.0 (0.7-1.5)</td>
<td>2.6 (2.3-3.7)</td>
<td>1.8 (1.3-2.4)*</td>
</tr>
<tr>
<td>VLDL-C, g/L</td>
<td>0.2 (0.1-0.2)</td>
<td>0.2 (0.2-0.3)</td>
<td>0.6 (0.3-1.1)*</td>
</tr>
<tr>
<td>Triglyceride, g/L</td>
<td>0.7 (0.4-1.2)</td>
<td>1.2 (0.7-1.5)</td>
<td>3.2 (2.4-5.8)*</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and VLDL-C, very-low-density lipoprotein cholesterol. Values are expressed as median (range).

*P<.05, †P<.01 by Mann-Whitney U test.

Results of platelet-collagen adhesion and thrombus volumes after 3-minute perfusions at a wall shear rate of 2600 seconds⁻¹ are given in the Figure. Platelet-collagen adhesion in both groups of patients was significantly higher than in the group of healthy individuals (70.7 [61.2 to 82.0] and 70.3 [66.4 to 81.0] in types IIa and IIb patients, respectively, versus 51.2 [44.5 to 68.6] in the group of healthy individuals; P<.05). All values are percent median [range].

Platelet adhesion was significantly correlated with plasma levels of total cholesterol, triglycerides, LDL-C, and VLDL-C (r=.59, r=.53, r=.48, and r=.55, respectively; P<.05). However, since all the lipid parameters are interrelated, we performed a multiple regression analysis to assess the independence of the relations. In this manner, platelet adhesion was exclusively associated with cholesterol and VLDL-C (β=0.47 and β=0.42, respectively; P<.05). There was no correlation between plasma fibrinogen or von Willebrand factor and platelet adhesion (P>.2).

The thrombus volume in type IIa and IIb patients was not different from the group of healthy individuals (P>.10). The fibrin deposition was negligible in the three groups (<1%).

Discussion

Many studies show that hyperlipoproteinemia is associated with platelet hyperactivity as measured by the platelet aggregation device. However, the clinical relevance (eg, thrombogenesis) of this finding is not known because platelet aggregation studies are performed with anticoagulated and nonflowing blood. A much more direct and relevant approach for investigating the influence of hyperlipoproteinemia on thrombogenesis is represented by perfusion studies, in which platelet-vessel wall and platelet-platelet interactions are studied under well-characterized blood flow conditions using nonanticoagulated blood. The thrombogenic effect of contraceptive drug treatment has been studied in this way.

Using this methodology, we have shown that platelet-collagen adhesion in flowing nonanticoagulated blood from patients with type IIa and type IIb hyperlipoproteinemia is significantly increased at a high arterial shear rate. The platelet-platelet interaction is apparently not affected by these metabolic disorders.

No studies have used this human method to evaluate the influence of hyperlipoproteinemia on platelet-vessel wall interactions. Badimon et al have recently shown that platelet adhesion and thrombus formation measured on de-endothelialized rabbit aortas at a high shear rate (2600 seconds⁻¹) were increased in hyperlipidemic rabbits. Tandon et al using the Baumgartner perfusion device, de-endothelialized rabbit aortas as a thrombogenic surface, arterial flow conditions, and an-
ticocagulated blood from patients with types IIa and IIb dyslipidemia, report that only patients with type IIb dyslipidemia have enhanced platelet adhesion. The platelet thrombus size was not significantly affected by the hyperlipoproteinemic state. The present results confirmed the latter findings, showing that platelet adhesion and not thrombus formation is increased in patients with type IIb hyperlipoproteinemia, but in a system making use of nonanticoagulated blood. In addition, we found that the platelet-collagen adhesion in nonanticoagulated blood from type IIa patients was enhanced as well.

The discrepancies between the human and rabbit studies are not due to differences in flow conditions. Both investigations were performed at arterial shear rate. Perfusion times were 5 and 20 minutes in the rabbit study, 30 minutes in the study performed by Tandon et al, and 3 minutes in the present work. We chose a short perfusion period because the first minutes of the thrombus formation are of greatest importance for the in vivo consequences. Furthermore, the highest differences of thrombus formation between normal and hypercholesterolemic rabbits are seen with the shortest perfusion periods. The disagreement between the human and rabbit studies is probably not due to differences in the nature of thrombogenic surfaces, since both Tandon et al and Badimon et al used subendothelium; we confirmed the finding of Tandon et al using nonprocoagulant collagen fibers. More presumably, differences are related to the different amounts of cholesterol in the diet, which resulted in plasma concentrations of 15 g/L total cholesterol in the rabbits compared to 3 to 4 g/L in the patients. Thus, the extremely high plasma cholesterol levels in the rabbits, which is not reached in humans, may explain this disagreement. It is interesting to note that Tandon et al showed that thrombus size was indeed increased when in vitro cholesterol-enriched platelets were used instead of platelets from hyperlipoproteinemic patients having a cholesterol-to-phospholipid ratio higher than those generally found in platelets from type IIa or IIb patients. Thus, these results suggest that the effect of hyperlipoproteinemia on thrombus formation is dependent on the level of hyperlipoproteinemia and is detectable at markedly high plasma lipid levels.

Explanation of the enhanced platelet-collagen adhesion at a high arterial shear rate remains largely undefined. Previous work has shown that platelet hyperreactivity in hyperlipoproteinemia could be due to the binding of lipoproteins (eg, LDL in type IIa and VLDL in type IIb) to platelet membranes. This may change the membrane fluidity, the enzymes involved in the synthesis of thromboxane A2, and/or the number of agonist receptors. Also, the sensitivity of cholesterol-rich platelets to thromboxane A2 appears to be increased. In our study, the enhanced platelet adhesion was apparently related to the plasma levels of both total cholesterol and VLDL-C.

Besides the effect of hyperlipoproteinemia on platelets themselves, other mechanisms may have played a role in the enhancement of the platelet adhesion. Reduction of erythrocyte deformability, which is seen in cholesterol-enriched erythrocytes, promotes platelet adhesion to subendothelium. von Willebrand factor, which strongly supports platelet adhesion at high wall shear rate, was probably not affected in these patients because its plasma levels did not differ between the groups of healthy subjects and patients. Likewise, there were no differences between patients and subjects with respect to fibrinogen plasma levels.

The increased platelet adhesion seen in types IIa and IIb hyperlipoproteinemic patients may be involved in the accelerated development of atherosclerosis in these patients, besides other major mechanisms than the accumulation of lipid in the vessel wall. The consequences of intensive lipid-lowering therapy on regression of platelet adhesion and coronary arteriosclerosis remain to be investigated.

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References

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