Effects of Abciximab on the Architecture of Platelet-Rich Clots in Patients With Acute Myocardial Infarction Undergoing Primary Coronary Intervention

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Background—Abciximab plus aspirin improves the TIMI 3 flow rate of the infarct-related artery in patients treated with either percutaneous coronary intervention or thrombolysis. The present study investigated whether the reperfusion efficacy of abciximab relates to modifications of clot architecture in patients admitted for acute myocardial infarction (AMI).

Methods and Results—A total of 23 AMI patients in the Abciximab before Direct angioplasty and stenting in Myocardial Infarction Regarding Acute and Long term follow-up (ADMIRAL) trial received, in a double-blind fashion, either abciximab (n=13) or placebo (n=10) before primary stenting. Viscoelastic (G' in dyne/cm²) and morphological (mean platelet aggregate surface area [SAG] in μm²) indexes of ex vivo platelet-rich clots (PRC) were assessed in a double-blind fashion before and after the bolus administration of abciximab or placebo. G' and SAG reflect the mechanical and morphological impact of activated platelets on the PRC fibrin network, respectively. Abciximab administration reduced G' by 63% (P=0.0001) and SAG by 65% (P=0.0007), and no effect was seen in the placebo group. These abciximab-related changes increased fibrin exposure as a consequence of the platelet-aggregate surface reduction and may have improved endogenous fibrinolysis. These effects were identified in all patients, independent of previous heparin administration.

Conclusions—Abciximab dramatically reduces platelet aggregate size and increases the fibrin accessibility of ex vivo PRC in AMI patients. These modifications could participate in the better coronary artery patency observed with abciximab. (Circulation. 2001;103:2328-2331.)

Key Words: myocardial infarction ■ fibrin ■ platelets ■ abciximab ■ thrombosis

The reperfusion efficacy of abciximab in the setting of acute myocardial infarction (AMI) was initially described by Gold et al., and it was confirmed recently in large trials, with full reperfusion achieved in 17% to 32% of patients. The French study, Abciximab before Direct angioplasty and stenting in Myocardial Infarction Regarding Acute and Long term follow-up (ADMIRAL), assessed the superiority of abciximab over placebo in AMI patients treated with primary percutaneous coronary intervention and stenting. The aim of the present work, a substudy of the ADMIRAL trial, was to investigate whether abciximab could modify the physical properties of ex vivo platelet-rich clots (PRC) in 23 patients admitted to the same center for AMI who were eligible for primary percutaneous coronary intervention. We previously reported that the PRC architecture in healthy volunteers was modified by the addition of abciximab in vitro, which led to a faster fibrinolysis rate.

In the present study, physical properties of PRC were assessed ex vivo by measuring the viscoelastic (G' in dyne/cm²) and morphological (platelet aggregate surface area [SAG] in μm²) properties of native, fully hydrated PRC from AMI patients randomized to receive, in a double-blind fashion, abciximab or placebo. G' and SAG reflect the mechanical and morphological impact of activated platelets on the clot fibrin network, respectively. The lower the values of G' and SAG, the more sensitive the fibrin network is to endogenous or therapeutic fibrinolysis. We hypothesized that modifications of clot physical properties in AMI patients...
could be induced by abciximab and participate in the complex mechanisms of reperfusion.

Methods

Selection of Patients and Study Design
All 23 patients were enrolled in the ADMIRAL trial at Pitié-Salpêtrière hospital with a diagnosis of AMI, as defined in the protocol (ischemic chest pain >30 minutes; onset of symptoms <12 hours; and ST elevation in ≥2 contiguous leads). All patients received aspirin (500 mg IV), heparin, and, in a double-blind fashion, a bolus (0.25 mg/kg) of either abciximab (n=13) or placebo (n=10) before primary percutaneous coronary intervention and stenting. A 12-hour continuous infusion (0.125 μg · kg⁻¹ · min⁻¹) of abciximab (or placebo) was immediately started.

Formation of PRC
Venous blood was drawn (1 volume of 0.13 mol/L citrate for 9 volumes of blood) immediately before and 10 minutes after the bolus administration. Both blood samples were obtained before the first contrast media injection and before heparin administration, except in 5 patients who received heparin (5000 IU) during transportation to the catheterization laboratory. All patients received aspirin before blood sampling. Platelet-rich plasma was obtained as previously described to reach an average final platelet count of 125 000 cells/μL.³ Adding 20 mmol/L CaCl₂ and 0.125 IU/mL thrombin (Enzyme Research Laboratories Inc) led to the formation of PRC.

Analysis of the Physical Properties of Ex Vivo PRC
As previously described,³ physical properties of PRC were assessed within 30 minutes of venous blood collection. All measurements were done in a blinded fashion regarding the treatment. Briefly, the rigidity index G’ (dyne/cm²) was measured; it reflects the mechanical retraction of the fibrin network by platelets.⁵ The PRC were labeled with fluorescein isothiocyanate (Sigma) and processed with a laser confocal system to generate a 3D reconstructed image of PRC architecture, which allowed the determination of the average fibrin/platelet aggregate surface area (SAG in μm²).⁵ ⁷

Statistical Measurements
Statistical analysis was performed with the StatView software package (Version 5.0, Abacus Concepts, Inc). Continuous variables were expressed as mean±SEM, and group differences were determined by ANOVA with the Bonferroni correction. An α level of 0.05 was accepted as significant.

Results

Patient Characteristics
Ten patients were randomly assigned to placebo (80% men; mean age, 67±3 years), and 13 received abciximab (85% men; mean age, 61±3 years). Infarct location was anterior in 40% and 50% of placebo and abciximab patients, respectively. The mean delay between the onset of symptoms and study drug administration trended to be shorter in abciximab patients than in placebo patients (270±70 versus 170±30 minutes, P=0.16). The first angiogram was always performed after the initiation of treatment, and TIMI 3 flow at first contrast media injection was seen in 10% and 23% of placebo and abciximab patients, respectively (P=0.77).

Abciximab and placebo patients had similar levels of fibrinogen (2.9±0.9 versus 3.1±1.2 g/L, respectively, P=0.55) and similar platelet counts (253 200±21 300 versus 231 500±12 500 platelets/μL, respectively, P=0.19).

Effect of Abciximab on PRC Properties
Confocal micrographs of PRC showed a heterogeneous structure with branching fibers alternating with dense platelet-rich areas (Figure 1A). These aggregates were made of a high concentration of fibrin fibers retracted by the platelet contractile force (Figure 1B).³ Boundaries of these aggregates were detected as shown in Figures 1A and 1B. In platelet-poor areas, individual fibers and branching fibers were easily visualized (Figure 1B).

A significant and positive correlation was found between G’ and SAG (r=0.6, P<0.01), indicating that patients with the largest aggregates displayed the stiffest clots as a consequence of a greater retraction of the fibrin fibers by activated platelets. There were no significant differences for G’ and SAG between placebo and abciximab patients before administration of the bolus (Figure 2). However, patients who received a bolus of heparin before reaching the catheterization laboratory (n=5) had a weaker PRC than patients who were free of heparin at the time of blood sampling (G’, 950±192 versus 2085±233 dyne/cm² in patients with and without heparin, respectively, P=0.01). These findings are related to the anticoagulant effect of heparin, which affects fibrin formation and leads to a looser conformation of the fibrin network made of fewer and thicker fibers (Figure 1C).⁸ However, platelet aggregate size (SAG) remained similar in patients with and without heparin (2478±418 versus 2754±283 μm², respectively, P=0.62).

Abciximab dramatically affected the physical properties of PRC: G’ decreased by 63% (from 1870±317 dyne/cm² at baseline to 689±139 dyne/cm² after abciximab) and SAG decreased by 65% (from 2522±330 μm² at baseline to 891±89 μm² after abciximab), but placebo had no significant effect on either G’ or SAG (Figures 1D, 1E, 1F, and 2). The correlation between G’ and SAG remained significant after administration of the bolus (r=0.59, P=0.024). A similar magnitude in the reduction of G’ and SAG was measured in abciximab patients who received heparin and those who did not (74±13% with heparin versus 64±5% without heparin, P=0.99, for G’; 71±5% with heparin versus 60±6% without heparin, P=0.21, for SAG).

Discussion
Abciximab administration in vitro can disaggregate PRC formed with the blood of healthy volunteers, improving fibrinolysis as a consequence of a better fibrin exposure to lytic agents within areas of platelet aggregates.⁵ The present study further demonstrates that abciximab alters the physical properties of PRC in AMI patients, and this may be one of the mechanisms for the effect of abciximab in restoring TIMI 3 flow when it is given with aspirin.

The administration of heparin and aspirin is associated with a TIMI 3 flow rate <13%.⁹ ¹⁰ Abciximab on top of aspirin and heparin can achieve TIMI grade 3 flow in 17% to 32% of patients,² ³ ⁴ and the rapid effect of abciximab on reperfusion suggests a direct action on the thrombus structure.¹ The major finding of this study is that abciximab in AMI patients dramatically affects the architecture of ex vivo PRC compared with placebo. These modifications primarily consist of an increase in fibrin exposure within platelet-rich areas. These abciximab-
related changes have been shown to increase the rate of fibrinolysis in vitro, and they offer a mechanistic explanation regarding why reduced doses of fibrinolytic agents may be successful in restoring flow in epicardial infarct arteries when combined with abciximab.3–5 Although heparin has been shown to affect fibrin formation and to abolish platelet contractile forces,8,11 abciximab efficacy in modifying PRC was independent of the effect of heparin on fibrin properties.

The PRC of AMI patients are 2-fold stiffer than the PRC of healthy volunteers formed under the same conditions, but the reductions of G9 and SAG reported here in abciximab-treated AMI patients are similar to those measured previously when abciximab (0.068 μmol/L) was added in vitro to platelet-rich plasma from healthy volunteers before the initiation of coagulation.5 Furthermore, pressure-driven permeation studies with a similar bolus of abciximab added to preformed PRC showed disaggregation of these PRC, resulting in a significant increase of fibrinolysis.5 Altogether, these data suggest that abciximab-related changes in PRC properties due to platelet disaggregation may improve endogenous fibrinolysis and coronary artery patency. However, in vivo thrombus formation is a complex phenomenon involving numerous intricate mechanisms that our experimental design cannot consider.

Figure 1. Micrographs of PRC in AMI patients. A, PRC displays platelet-poor areas made of branching fibers (arrows) that are organized in a 3D network alternating with platelet-rich areas made of retracted fibrin fibers, surrounded here by contours that are automatically detected. B, In some cases, platelet-poor areas look like meandering channels with a loose fibrin network (arrows). C, Heparin without abciximab leads to the formation of thicker fibers (arrow and arrowhead) that are organized in a looser network compared with PRC formed without heparin (A). D, Placebo has no effect on the overall architecture (A is before and D after placebo). E, In vivo administration of abciximab leads to smaller platelet-rich areas and higher fibrin exposure (B is before and E after abciximab). F, Effect of abciximab in patients pretreated with heparin (C is before and F after abciximab). Bar=10 μm.

Figure 2. Changes in viscoelastic (□) and morphological (■) properties of PRC in patients with AMI receiving either placebo or abciximab before primary stenting and percutaneous coronary intervention. Unlike placebo, abciximab infusion significantly (*) lowered G9 by 63% and SAG (S.ag) by 65%, indicating weaker PRC with smaller platelet aggregates.
In conclusion, abciximab treatment in AMI patients limits platelet aggregate formation, promotes fibrin exposure in ex vivo PRC, and may account for the improved culprit artery patency when compared with placebo.

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