Administration of Abciximab During Percutaneous Coronary Intervention Reduces Both Ex Vivo Platelet Thrombus Formation and Fibrin Deposition
Implications for a Potential Anticoagulant Effect of Abciximab

George Dangas, Juan J. Badimon, Barry S. Coller, John T. Fallon, Samin K. Sharma, Richard M. Hayes, Perwaiz Meraj, John A. Ambrose, Jonathan D. Marmur

Abstract—Abciximab (c7E3 Fab, ReoPro), a platelet glycoprotein (GP) IIb/IIIa inhibitor, decreases acute ischemic complications after percutaneous coronary interventions. Recently, abciximab was shown to decrease thrombin generation in vitro in a static system. To assess whether abciximab can decrease fibrin formation in blood from patients, we quantified both platelet thrombi and fibrin deposition by using an ex vivo flow chamber model. We prospectively studied 18 consecutive patients who underwent percutaneous interventions for unstable coronary syndromes. Blood was perfused directly from the patient through an ex vivo perfusion chamber at a high shear rate, thus mimicking mildly stenosed coronary arteries. Perfusion chamber studies were performed when patients were being treated with heparin plus aspirin before the procedure (baseline) and then repeated after the procedure, when patients were on either aspirin plus heparin alone (group 1, no abciximab, control) or aspirin plus heparin plus abciximab (group 2, abciximab treated). Each patient served as his or her own control. Specimens were stained with combined Masson’s trichrome–elastin and antibodies specific for fibrinogen, fibrin, and platelet GP IIIa. Total thrombus area and areas occupied by platelet aggregates and fibrin layers were quantified by planimetry. Group 1 demonstrated no significant change in thrombus area before versus after the procedure; in contrast, treatment with abciximab reduced total thrombus area by 48% in group 2 (after the procedure versus baseline, \( P < 0.01 \)). This decline was due to significant reductions in both platelet aggregates (55%, \( P < 0.005 \)) and fibrin layers (45%, \( P = 0.03 \)). The addition of abciximab to heparin and aspirin in patients undergoing coronary interventions significantly decreases ex vivo thrombus formation on an injured vascular surface. Treatment with abciximab appears to reduce both the platelet and the fibrin thrombus components. This finding supports a potential role for GP IIb/IIIa receptor blockade in decreasing fibrin formation in addition to inhibition of platelet aggregation. Thus, potent inhibitors of GP IIb/IIIa may also act as anticoagulants. (Arterioscler Thromb Vasc Biol. 1998;18:1342-1349.)

Key Words: angioplasty thrombosis platelets

Percutaneous coronary interventions (PCIs) are associated with vessel wall injury at the treatment site. The local response is mediated by exposure of deep components of the arterial wall and stimulation of platelet adhesion and aggregation, thrombin generation, and fibrin deposition.\(^1\)-\(^3\) The developing thrombus is highly thrombogenic and acts as a further stimulus to platelet deposition, thrombin generation, and fibrin formation.\(^4\)\(^5\) The clinical implications of intracoronary thrombosis after PCI are important because most short-term procedural complications are due to thrombosis; in addition, mural thrombus formation after PCI has been implicated in the pathogenesis of atherosclerosis and restenosis.\(^7\)

Activation of the platelet glycoprotein (GP) IIb/IIIa integrin initiates high-affinity binding of von Willebrand factor or fibrinogen, which is the final common pathway of platelet aggregation regardless of the initial thrombogenic stimulus.\(^8\) The addition of the mouse-human chimeric monoclonal antibody abciximab (c7E3 Fab, ReoPro), which blocks GP IIb/IIIa and the related \( \alpha_v \beta_3 \) receptor,\(^9\)\(^10\) to a regimen of aspirin and heparin decreases early postprocedural complications of PCI and improves the short-term clinical outcome.\(^10\) Platelet GP IIb/IIIa receptor blockade with abciximab inhibits platelet aggregation.\(^11\)\(^12\) Recently, abciximab was also shown to decrease thrombin generation in a static system.\(^13\) The blockade of GP IIb/IIIa by abciximab was primarily responsible for the decrease in thrombin generation, but the blockade of platelet \( \alpha_v \beta_3 \) receptors by abciximab probably also contributed to the effect. These data raise the possibility that abciximab may also decrease thrombin generation in vivo as one of its mechanisms of action.
STUDY PROTOCOL

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission to the General Clinical Research Center of Mount Sinai</td>
<td>Admission to the General Clinical Research Center of Mount Sinai</td>
</tr>
<tr>
<td>Begin heparin: 5000 U bolus and 1000 U/hr infusion (target aPTT 60-85 sec)</td>
<td>Begin heparin: 5000 U bolus and 1000 U/hr infusion (target aPTT 60-85 sec)</td>
</tr>
<tr>
<td>Baseline preprocessing perfusion chamber study on aspirin plus heparin</td>
<td>Baseline preprocessing perfusion chamber study on aspirin plus heparin</td>
</tr>
<tr>
<td>Discontinue heparin 1 hour prior to the procedure</td>
<td>Discontinue heparin 1 hour prior to the procedure</td>
</tr>
<tr>
<td>Begin abciximab 20 minutes prior to intervention 0.25 mg/kg bolus over 5 min and 100 mcg/min 12 hr infusion</td>
<td>Begin abciximab 20 minutes prior to intervention 0.25 mg/kg bolus over 5 min and 100 mcg/min 12 hr infusion</td>
</tr>
<tr>
<td>Percutaneous coronary intervention (target ACT 300 sec) Begin weight adjusted heparin (10 U/kg) postprocedure</td>
<td>Percutaneous coronary intervention (target ACT 300 sec) Begin weight adjusted heparin (10 U/kg) postprocedure</td>
</tr>
<tr>
<td>2-hour postprocedure perfusion chamber study on aspirin plus heparin</td>
<td>2-hour postprocedure perfusion chamber study on aspirin plus heparin plus abciximab</td>
</tr>
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</table>

Figure 1. Study protocol. C indicates perfusion chamber study; ACT, activated clotting time.

To test the hypothesis that abciximab may exert an anti-coagulant effect, we first modified our ex vivo flow chamber model so that we could independently assess both platelet thrombus formation and fibrin deposition, the latter being an indicator of thrombin generation. In this ex vivo perfusion chamber system, the thrombogenic substrate (severely damaged porcine aorta) is exposed to flowing human blood and the rheological conditions are standardized, allowing the evaluation of blood thrombogenicity.14-16 We studied PCI patients treated with and without abciximab by passing blood directly from the patient through the flow chamber. We report that the administration of abciximab to patients undergoing PCI significantly reduces fibrin deposition as well as platelet thrombus formation in this ex vivo model.

Methods

All patients transferred to the Mount Sinai Hospital, New York, NY, for PCI after diagnostic angiography performed at the City Hospital Center at Elmhurst, Queens, from November 1995 to May 1996 were eligible for enrollment in this study. Inclusion criteria were the presence of an unstable coronary syndrome or an angiographically high-risk lesion (definition below). Only patients with native coronary artery lesions of ≥70% diameter stenosis were included. Exclusion criteria included restenotic lesions, acute myocardial infarction (primary or rescue PCI), thrombocytopenia, bleeding disorders, and inability to obtain informed consent. Patients were transferred directly to the General Clinical Research Center of the Mount Sinai Medical Center where they were enrolled into the study protocol (Figure 1). The study was approved by the Institutional Review Board, and all enrolled patients gave written, informed consent.

Definitions

An unstable coronary syndrome was defined as postinfarction, refractory, or rest angina (Braunwald classes II or III, B or C),17 High-risk lesions were defined as complex lesions or lesions of type B2 or C according to the American Heart Association/American College of Cardiology classification.8,9 Complex lesions were defined as those with irregular borders or overhanging edges, with or without proximal or distal intracoronary filling defects (Ambrose criteria).18

Study Protocol

Patients were divided into 2 groups (Figure 1): group 1 comprised patients treated with conventional therapy (heparin plus aspirin) during the PCI, and group 2 comprised patients treated with abciximab in addition to conventional therapy. The decision to administer abciximab was at the discretion of the operator.

All patients underwent baseline (preprocedure) and postprocedure perfusion studies to evaluate ex vivo thrombus formation. All baseline perfusion studies were performed <24 hours before the PCI, while the patients were on aspirin plus heparin. Heparin had been started ≥12 hours earlier as a 5000-U bolus plus 1000 U/hr infusion, with subsequent adjustment to a target activated partial thromboplastin time (aPTT) of 60 to 85 seconds. Postprocedure perfusion studies were performed 2 hours after the PCI, while the patients were receiving either aspirin plus heparin (group 1) or aspirin plus heparin plus abciximab infusion (group 2).

Revascularization Procedure

Either balloon angioplasty or elective intracoronary stent implantation was performed in all patients by using the transfemoral approach with an 8F arterial sheath. All stents were deployed with high pressure (16 to 18 atm for 30 seconds, balloon-to-artery ratio of 1.0) without intravascular ultrasound guidance. The balloon-to-artery ratio during balloon angioplasties was 1.0 to 1.1 and the inflation pressure 6 to 8 atm for 100 seconds. Procedural success was defined as <30% residual diameter stenosis and absence of dissection or major complications (ie, death, infarction, or need for bypass surgery). The preprocedure heparin infusion was stopped 1 hour before the PCI. Heparin was administered during the procedure as repeated boluses to maintain the target activated clotting time of >300 seconds. After completion of the intervention, all patients received a weight-adjusted heparin infusion (10 U · kg⁻¹ · h⁻¹). Group 2 patients received abciximab administered as a 0.25 mg/kg IV bolus over 5 minutes plus a 10 µg/min infusion for 12 hours, starting 20 minutes before the PCI. A single operator (J.D.M.) performed all procedures.

Ex Vivo Perfusion Chamber

The perfusion chamber system has been described elsewhere.1,14,15,20 It consists of a cylindrical flow channel (1-mm diameter, 2-cm length) that allows the flowing blood, pumped directly from the patient, to flow over the exposed thrombogenic substrate. Local flow conditions mimicking mild arterial stenosis14,15,20 were kept constant in all experiments: a shear rate of 1690 s⁻¹, a Reynolds’ number of 60, excellent intraobserver reproducibility in the determination of thrombus formation with this model has been previously reported (r=0.95).15

Thrombogenic Substrates

Fresh-frozen porcine aortic tunica media was surgically prepared (25×10-mm sections) to simulate the degree of severe arterial injury induced by PCI, as previously described.1,14,20 Segments of the aorta were prepared by first removing excess adventitia and then, after longitudinally opening the aorta, peeling off the intima together with a thin portion of the underlying media, thereby exposing the deeper components of the arterial wall media (both matrix and cellular elements) to the flowing blood. Segments were stored at −20°C in 0.1 mol/L NaCl and 0.01 mol/L NaPO₄ (pH 7.4). The surface of this fresh-frozen preparation exposes deep components of arterial media (ie, collagen types III and IV, basement membrane components, fibronectin, and smooth muscle cells) to the flowing blood. The thrombogenic reaction formed on the porcine tunica media preparation is very similar to that formed on human arterial segments containing lipid-rich plaques.3 Previous studies indicated that murine 7E3 does not react with porcine platelets (B.S.C., unpublished data, 1997) and that abciximab does not react with porcine endothelial cell αvβ₃.21

Perfusion Studies

During each perfusion study, blood was circulated through 3 chambers connected in series. The perfusion chamber system was connected with polyethylene tubing (1-mm diameter) to the intravenous
line and to a peristaltic pump (Masterflex model 7013, Cole-Palmer Instruments), positioned distal to the chambers, and flushed with 0.9% NaCl for 30 seconds. With a tourniquet in place, a 20G cannula was inserted into an antecubital vein of the patient, and then the tourniquet was immediately removed. The first 10 mL of blood was discarded, and then the ensuing blood was passed directly from the patient through the chamber system for 5 minutes, after which the chambers were flushed with 0.9% NaCl for 1 minute under the same rheological conditions. The perfused substrates were then removed from the chambers, placed in formalin at 4°C for 48 to 72 hours, and then processed for immunocytochemistry and light microscopy. All 50 mL of blood was discarded after perfusion through the chamber system, and no blood was returned to the patient.

Evaluation of Thrombus Formation

Thrombus is formed along the entire length of the exposed substrate (equal to the window of the flow channel [2 cm]). Thus, the thrombus cross-sectional area is a reliable reflection of total thrombus. As previously described, 1,2 2-mm sections were cut from each formalin-fixed specimen from the proximal, middle, and distal thirds of the exposed surface and embedded in paraffin. Sections (5 μm) were prepared and stained with (1) combined Masson’s trichrome—elastin stain (CME), which stains total thrombus and does not distinguish platelets from fibrin; (2) a rabbit polyclonal anti-human fibrinogen antibody (A080, DKA) at 3.6 μg/mL, which reacts with fibrin II polymer but not with fibrinogen; and (4) a murine monoclonal anti-fibrin antibody (NYBT2G1, Accurate Chemical & Scientific Corp) at 1 μg/mL, which reacts with fibrin II polymer but not with fibrinogen. The sections were then processed for immunocytochemistry and light microscopy. All 50 μL of blood was discarded after perfusion through the chamber system, and no blood was returned to the patient.

Table 1. Clinical and Angiographic Characteristics and Laboratory Values

<table>
<thead>
<tr>
<th></th>
<th>Group 1: No Abciximab (n=9)</th>
<th>Group 2: Abciximab Treated (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62 ± 9</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Males</td>
<td>8 (89)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (33)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Refractory angina</td>
<td>2 (22)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Rest angina</td>
<td>3 (33)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Postinfarction angina</td>
<td>5 (56)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>AHA/ACC type B2/C lesions</td>
<td>1 (11)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Complex lesions</td>
<td>4 (44)</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>1 (11)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38 ± 5</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Platelet count, ×10^11/mL</td>
<td>217 ± 44</td>
<td>270 ± 70</td>
</tr>
<tr>
<td>aPTT, seconds</td>
<td>71 ± 17</td>
<td>77 ± 13</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>384 ± 39</td>
<td>421 ± 39</td>
</tr>
<tr>
<td>Postprocedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36 ± 3</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Platelet count, ×10^11/mL</td>
<td>225 ± 60</td>
<td>218 ± 48</td>
</tr>
<tr>
<td>Activated clotting time, s</td>
<td>287 ± 25</td>
<td>290 ± 23</td>
</tr>
</tbody>
</table>

AHA/ACC denotes the American Heart Association/American College of Cardiology lesion classification. None of the differences between the groups was statistically significant. Continuous variables are given as mean ± SD and categorical variables as n (%).

Results

Study Population

Of 48 patients transferred for PCI during the study period, 27 were excluded because of acute myocardial infarction (n = 10), restenosis (n = 7), Braunwald class I angina (n = 8), or inability to obtain informed consent (n = 2). Three of the remaining 21 eligible patients were subsequently excluded (cancellation of PCI owing to femoral pseudoaneurysm at the site of the diagnostic catheterization in 1 case, bleeding peptic ulcer in another, and performance of bypass surgery in the third).

There were no significant differences between the 2 groups of patients with respect to their clinical and angiographic characteristics (Table 1). Hematocrit, platelet count, and aPTT during the baseline perfusion studies were also similar in the 2 groups. Procedural heparin doses were 9444 ± 1667 U in group 1 versus 7389 ± 2497 U in group 2 (P = 0.06), and the mean procedural activated clotting time was 434 ± 64 seconds in group 1 versus 349 ± 57 seconds in group 2 (P = 0.02). Two group 1 and 4 group 2 patients were treated with stents and received the first dose of ticlodipine (250 mg) at the time of the procedure. After the procedure, there were no significant differences in the values of hematocrit, platelet count, or activated clotting time (Table 1). There was no significant difference between the 2 groups with respect to the duration of the procedure or the amount of contrast material used. All
procedures were successful, and all patients remained asymptomatic and were discharged to home 1 to 2 days after the PCI without any complications.

**Quantification of Thrombus Formation**
The total thrombus formation results for groups 1 and 2 both before and after the procedure are given in Table 2. There were no significant differences in the mean thrombus formation between the 2 groups in the baseline (preprocedure) perfusion studies, at which time both groups were receiving the same treatment (aspirin plus heparin), but there was a trend toward greater thrombus area in group 2 versus group 1 \( (P=0.08) \). After the procedure, mean total thrombus formation in group 1 patients was increased by 28%, but this change was not significantly different compared with baseline \( (P=0.2) \). In contrast, the mean postprocedure total thrombus formation in group 2 patients, who were receiving abciximab in addition to heparin and aspirin, was 48% less than the mean preprocedure value in this group \( (P<0.01) \) and 43% less than the mean postprocedure value in group 1 patients \( (P<0.01) \).

The changes in total ex vivo thrombus area values between the preprocedure and postprocedure studies in individual patients are presented in Figure 3. Only 2 of 9 patients in group 1 had decreases in total thrombus area, whereas 7 of 9 had increases. In contrast, 7 of 9 patients in group 2 had decreases in total thrombus area. Thrombus changes in patients who received a single dose of ticlopidine (pre-stent placement) between the 2 perfusion studies are also shown in Figure 2. When these patients were excluded from analysis, the abciximab-treated group still demonstrated a 10 000±8291 \( \mu \text{m}^2 \) decrease in total thrombus area in the postprocedure compared with the preprocedure perfusion experiment, contrasted with a 4377±5432 \( \mu \text{m}^2 \) increase in thrombus area in the postprocedure compared with the preprocedure perfusion experiment in the control group \( (P<0.01) \).

Characteristic examples of ex vivo thrombus formation in individual patients are shown in Figure 3 (CME and fibrinogen stains). In the preprocedure study of group 1 patient (ie, no abciximab), thrombus covered the entire exposed surface, with a number of areas showing platelet aggregates propagating in irregular accumulations (IA and IC). After the procedure, thrombi were larger and more confluent compared with those at baseline (cf IB and ID versus IA and IC). In addition, the fibrinogen stain was more intense on the surfaces of platelet thrombi as well as within the body of the large platelet thrombus (ID versus IC). The baseline study of group 2 patient (IIA and IIC) was very similar to that of the group 1 patient. In the postprocedure sample from the group 2 patient, who received abciximab treatment, less thrombus growth was observed in comparison with baseline (cf IIB and IID versus IIA and IIC) and the group 1 patient after the procedure (cf IIB and IID versus IB and ID).

**Quantification of Platelet and Fibrin Deposition**
As shown in Figure 3, the thrombus appeared to be composed of irregularly shaped platelet aggregates and a fibrin layer.

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**TABLE 2. Total Thrombus Area Before (Baseline) and After the Intervention**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=9)</th>
<th>Group 2 (n=9)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline value</td>
<td>11 454±4771</td>
<td>16 575±6856</td>
<td>0.08</td>
</tr>
<tr>
<td>Postprocedure value</td>
<td>14 979±4982</td>
<td>8603±4696*</td>
<td>0.008†</td>
</tr>
<tr>
<td>Change in total thrombus (post−pre)</td>
<td>+3525±6315</td>
<td>−7971±7575</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Variables are given as mean±SD.
*\( P<0.05 \) compared with baseline; †\( P<0.01 \) by ANCOVA controlling for baseline between-group differences.
The CME stain outlined the areas of thrombus formation but did not differentiate between regions of platelets versus that of fibrin. The fibrinogen antibody identified a variably dense layer of thrombus on the surface of the denuded area and showed weak staining of the platelet aggregates.

To investigate the effects of abciximab on these thrombus components, we used antibodies specific for platelets and fibrin (7H2 and anti–ββ5–42, respectively) (Figure 4). The anti-GP IIIa platelet antibody (7H2) identified platelets and platelet aggregates, and the fibrin-specific antibody stained the linear thrombus layer over the denuded surface but did not stain the platelet aggregates.

Quantitative results of the areas of platelet aggregates and fibrin layers in the 2 groups are shown in Table 3. In specimens derived from patients who did not receive abciximab (group 1), platelet aggregates and fibrin layers were increased after the procedure compared with baseline, but the differences were not statistically significant. In contrast, both platelet aggregates and fibrin layers were significantly decreased in the postprocedure versus preprocedure specimens derived from patients treated with abciximab (group 2), with platelet aggregates having been reduced by 55% ($P=0.005$) and fibrin layers by 45% ($P=0.03$). An ANCOVA controlling for between-group differences at baseline showed that group 2 had a smaller platelet aggregate area ($P=0.01$) and fibrin layer area ($P=0.05$) in the postprocedure studies when compared with group 1. There were no significant relationships between activated clotting time values or heparin dose, and either platelet aggregate area or fibrin layer area.

**Figure 3.** Representative photomicrographs of porcine media exposed to flowing blood from a group 1 patient treated with heparin and aspirin only (I) and a group 2 patient treated with heparin, aspirin, and abciximab (II). A and C are preprocedure (baseline) and B and D are postprocedure. Sections in A and B were stained with CME, whereas sections in C and D were stained for fibrinogen. Two morphologically distinct thrombus components are present: irregularly shaped platelet aggregates (light brown in C and D) and uniform, fibrinogen-positive mural thrombus layer (dark brown in C and D). Sections from the group 1 patient, who did not receive abciximab, show considerable postprocedure thrombus growth (IB and ID). However, a decrease in postprocedure compared with baseline thrombus area was seen with both stains in sections from the group 2 patient, who received abciximab after the intervention (IIIB and IID). Original magnification ×100; in C and D, brown indicates peroxidase developed with 3,3'-diaminobenzidine.

**Discussion**

The antiplatelet effect of GP IIb/IIIa blockade has been previously evaluated. Sakariassen and coworkers demonstrated in vitro that platelet aggregation on human artery subendothelium under high-shear-rate conditions could be inhibited by monoclonal antibodies against GP IIb/IIIa. The effect of abciximab on platelet aggregation in patients undergoing PCI has been evaluated by Konstantopoulos et al., who demonstrated a 50% reduction in shear stress–induced platelet aggregation with abciximab, and by Turner et al,
who demonstrated >50% inhibition of platelet aggregation with abciximab, as determined by real-time analysis with an epifluorescence videomicroscopy system. None of these studies assessed the independent contributions of platelets and fibrin to thrombus formation, and neither of the last 2 studies included direct visualization of mural thrombus under light microscopy. In our study, blood was passed directly from the patient into the experimental chamber, and we quantified total thrombus formation (Figure 3 and Table 2) as well as its 2 morphological components, platelet aggregates and the fibrin layer (Table 3), by direct visual assessment. Quantification of total thrombus revealed that abciximab significantly inhibited thrombus formation on the exposed vascular surface compared with conventional anti-thrombotic therapy in the control group.

Prior experimental studies have evaluated the impact of the absence of the GP IIb/IIIa receptor or of GP IIb/IIIa inhibition on fibrin deposition in flow system models, with controversial results. Weiss et al described increased fibrin deposition when the blood of patients with Glanzmann’s thrombasthenia was passed through a flow chamber, whereas Cadroy et al found decreased fibrin(ogen) deposition when nonhuman primates were treated with a GP IIb/IIIa antagonist. It is not clear which factor accounts for the discrepancies in the results from these studies, but technical differences and differences between platelets lacking the GP IIb/IIIa receptor on an inherited basis and those whose GP IIb/IIIa have been inhibited pharmacologically may account for the observed differences.

We found that abciximab decreases total thrombus formation by reducing both platelet aggregates and the fibrin layer of the thrombus (Table 3). The reduction in fibrin-positive thrombus suggests a decrease in fibrin deposition and thus, most likely a decrease in thrombin generation and activity as a result of abciximab therapy. Because activated platelets facilitate thrombin generation by providing a surface on which coagulation reactions occur efficiently and perhaps other mechanisms, the reduction of the total platelet mass available for thrombin formation with abciximab may result in decreased thrombin generation or activity. In addition to this quantitative effect, abciximab can decrease thrombin generation supported by platelets when defibrinated plasma is treated with tissue factor, indicating a possible qualitative effect of abciximab on thrombin generation, perhaps due to a decrease in platelet microparticle formation. Because platelets contain platelet factor 4, which can be released from activated platelets and neutralize heparin, it is also possible that abciximab’s ability to decrease fibrin deposition results from decreased release of platelet factor 4, leading to a relative augmentation of heparin’s action.

The effects of abciximab on thrombin generation and fibrin deposition may be relevant to the mechanism of action underlying its clinical benefit in decreasing ischemic complications after PCI. It also supports the concept that powerful antiplatelet agents, such as abciximab, may have antiagulant as well as antiplatelet effects. This idea has been independently suggested on the basis of the ability of abciximab to prolong the activated clotting time, regardless of whether the abciximab was administered to patients or added to heparinized blood in vitro. It is particularly impressive that abciximab can apparently reduce fibrin deposition at the site of thrombus formation even while the patients are receiving heparin by intravenous infusion. This result is consistent with ex vivo and in vivo data including the activated clotting time and supports our hypothesis that abciximab is working through a platelet-dependent mechanism rather than a fluid-phase coagulation mechanism. Nonetheless, the precise mechanism through which abciximab decreases fibrin deposition is unknown. This effect may be

| TABLE 3. Changes of the Two Morphological Components of the Ex Vivo Thrombus Before (Baseline) and After the Intervention |
|---|---|---|
| | Platelet Aggregate (PA) Area, μm² | Fibrin Layer (FL) Area, μm² |
| | Baseline | Postprocedure | ΔPA (Post−Pre) | Baseline | Postprocedure | ΔFL (Post−Pre) |
| Group 1 (no abciximab) (n=9) | 4405±2459 | 5204±1876 | +799±3582 | 7049±4457 | 9774±3374 | +2725±5043 |
| Group 2 (abciximab treated) (n=9) | 4843±1574 | 2167±1535* | −2676±2076 | 11 732±5764 | 6436±3813* | −5295±6206 |
| P value (between groups) | 0.7 | 0.002 | 0.02 | 0.07 | 0.07† | 0.008 |

* Variables are given as mean±SD.
† P<0.05 compared with baseline; † P=0.05 by ANCOVA controlling for baseline differences.
due to interdiction of a platelet phospholipid surface or direct attenuation of thrombin generation or activity.

There were selected patients in group 1 who demonstrated increases in thrombus formation after compared with before the preprocedure, which is consistent with previous findings that in some patients, PCI may lead to increased thrombogenicity. However, the overall changes in group 1 were not statistically significant, demonstrating that the PCI itself and the periprocedural change in the dosage of heparin had little overall effect on thrombus formation compared with that of the preprocedure regimen. The use of a single dose of ticlopidine in patients treated with stents did not appear to affect the results (Figure 2), because exclusion of these patients from analysis did not affect the statistical significance of the observed changes in thrombus formation. This finding was expected, given that ticlopidine has been reported to not exert significant antiplatelet effect within 24 hours of initiation of therapy.

**Study Limitations**

Because administration of abciximab in this study was directed by the physician, abciximab-treated patients were judged to be at higher risk for ischemic complications than the other group. In fact, the abciximab-treated group had a higher incidence of multivessel disease, complex lesions, diabetes, and rest angina (Table 1), although none of these differences were statistically significant between the 2 groups. Baseline ex-vivo thrombus formation between the 2 groups was not statistically different, despite a trend toward enhanced thrombus formation in group 2. Thus, the group treated with abciximab may have had a trend toward a greater predisposition to thrombus formation (Table 2). In addition, patients in group 2 compared with those in group 1 received less heparin and had lower activated clotting times during the PCI, which may have enhanced the predisposition to fibrin formation in group 2 patients during the PCI; the postprocedure values, however, were similar (Table 1).

Abciximab does not cross-react with porcine α,β, receptors. Thus, if the blockade of human medial smooth muscle cell α,β, by abciximab contributes to its antithrombotic effects in humans, then it is possible that our model underestimates the antithrombotic effects of abciximab. Although patients treated with stents received a single dose of ticlopidine between the 2 perfusion chamber studies, it is unlikely that this affected the results. Moreover, exclusion of these patients from the analysis did not affect the results (Figure 2). The efficacy of antiplatelet and antithrombotic medications may differ in dynamic compared with static flow conditions. For this reason, simulation of the specific in vivo blood flow characteristics that exist in a coronary artery after a PCI should be important for evaluation of the efficacy of antithrombotic therapy with a perfusion chamber system. Because postprocedure residual luminal diameter stenoses of 19% to 34% have been reported at the site of coronary stenting or angioplasty, the shear rate applied in flowing blood during our experiments was adjusted to simulate the rheology of a mildly stenosed arterial lumen. However, in vivo platelet reactivity to damaged human arterial surfaces may be different from platelet reactivity to the thrombogenic substrate used in this study.

Thrombus formation along the course of the chamber system may be variable. We attempted to minimize the sampling error by obtaining multiple cross sections from each specimen and by performing the perfusion studies in triplicate both before and after PCI. To minimize the variability in the preparation of the thrombogenic substrates, we had a single investigator prepare all porcine aortic substrates and perform the perfusion studies. The morphometric analyses were done in a blinded fashion after completion of the study.

**Implications**

Our data provide insight into the potential mechanisms through which abciximab produces the beneficial effects observed in coronary interventional trials. Treatment with abciximab reduces total thrombus formation not only as a result of inhibition of platelet aggregation but also due to decreased fibrin deposition. This potential anticoagulant mechanism of abciximab may be important in conceptualizing antithrombotic strategies.

**Acknowledgments**

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**References**


Administration of Abciximab During Percutaneous Coronary Intervention Reduces Both Ex Vivo Platelet Thrombus Formation and Fibrin Deposition: Implications for a Potential Anticoagulant Effect of Abciximab

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