Pharmacodynamics of Chimeric Glycoprotein IIb/IIIa Integrin Antiplatelet Antibody Fab 7E3 in High-Risk Coronary Angioplasty

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Background Thrombosis has been implicated as central to the clinical complications of coronary angioplasty (PTCA). Chimeric monoclonal 7E3 Fab (c7E3 Fab) is the first of a new class of antiplatelet drugs directed at the platelet glycoprotein IIb/IIIa integrin. This study was performed to determine the pharmacodynamics of c7E3 Fab administration during PTCA and to gain an initial clinical experience with this novel agent.

Methods and Results The study was a multicenter, open-label, dose-escalation study conducted in two stages. Enrollment included 56 patients scheduled for elective PTCA who were estimated to be at moderate to high risk of sustaining ischemic complications. All patients were given aspirin and heparin. The study drug was given at least 10 minutes before PTCA. In stage 1, increasing bolus doses of c7E3 Fab were given to 15 patients; a bolus dose of 0.25 mg/kg was found to result in blockade of >80% of the receptors and reduce platelet aggregation to <20% compared with baseline, establishing this dose as that necessary to sufficiently suppress platelet activity. In stage 2, additional c7E3 Fab was administered by continuous infusion to 32 patients for progressively longer periods of time (up to 24 hours) to confirm that platelet inhibition could be maintained with prolonged drug infusion. Also, 9 patients otherwise meeting entry criteria were given placebo. There were no thrombotic events among patients receiving c7E3 Fab. Overall procedural and clinical success and complication rates as well as rates of bleeding were statistically similar among groups. However, minor bleeding was more frequent with administration of the active drug.

Conclusions The novel antiplatelet agent c7E3 Fab can be administered during PTCA in combination with aspirin and heparin. Suppression of platelet activity is dose dependent and can be maintained for up to 24 hours. Further evaluation will be required to determine the extent of improvement in ischemic complication and restenosis rates and to provide additional insight into the safety profile of this potent monoclonal platelet antibody. (Circulation. 1994;90:1757-1764.)

Key Words • angioplasty • platelets • antibodies

Despite advances in the technology of percutaneous transluminal coronary angioplasty (PTCA), this procedure remains limited by the persistent 4% to 8% incidence of abrupt closure and 28% to 56% incidence of restenosis.1-3 Uncontrolled platelet deposition has been implicated in the processes of both abrupt closure and restenosis after PTCA.4-7 Abrupt closure remains a major clinical problem and accounts for a substantial portion of the morbidity, mortality, and emergency coronary bypass surgery accompanying PTCA.1,2

The sequence of events leading to thrombosis is initiated by the adhesion of platelets to sites of vascular injury. After adhesion, platelets become activated by local agonists, including epinephrine, ADP, collagen, serotonin, and thrombin. With activation, glycoprotein IIb/IIIa (GP IIb/IIIa) receptors on the platelet surface assume an active conformation. These receptors, typically numbering 50 000 to 100 000 per platelet, can then bind circulating fibrinogen, von Willebrand factor, and other adhesive proteins, resulting in platelet aggregation and ultimately thrombosis.8-10

As originally developed, the monoclonal antibody 7E3, specific for the human platelet GP IIb/IIIa integrin, was entirely murine in composition. In vitro, this agent completely inhibited platelet aggregation and, in animal models of angioplasty injury and thrombolysis, prevented thrombosis and augmented the activity of thrombolytic agents.11-14 Phase 1 clinical trials demonstrated a satisfactory safety and efficacy profile in the settings of unstable angina and elective PTCA.15,16 Because of concerns about immunogenicity, the derivative product chimeric monoclonal 7E3 Fab (c7E3 Fab) was created via genetic reconstruction. This new molecule consisted of the mouse-derived variable regions from the original molecule linked to the constant regions derived from human immunoglobulin IgG.

In this study we sought to gain an initial experience with c7E3 Fab in a clinical setting of potential thrombosis. Data accrued from this preliminary experience then served as the pharmacodynamic basis for dosing of c7E3 Fab in the Evaluation of c7E3 Fab in the Prevention of Ischemic Complications (EPIC) trial.17,18 Specifically, the purpose of this study was to develop a rational...
Chimeric 7E3 Fab Study Drug

Chimeric 7E3 Fab consists of the variable regions of the murine Fab fragment of the original 7E3 immunoglobulin reconstituted with human constant regions (Fig. 1). The study drug was supplied by Centocor, Inc, as a sterile, nonpyrogenic solution containing 2 mg of c7E3 Fab per mL of 0.15 mol/L sodium chloride and 0.01 mol/L sodium phosphate.

PTCA Procedure

Patients were pretreated with aspirin (325 mg) before the PTCA procedure. Heparin was given to achieve an activated clotting time of >300 seconds or activated partial thromboplastin time of >150 seconds before balloon inflation. Either active study drug or placebo was given at least 10 minutes before balloon inflation. PTCA was otherwise performed in the usual fashion. After the procedure, heparin was continued at the discretion of the investigator. Sheaths were removed 4 to 5 hours after discontinuation of heparin and a minimum of 6 hours after the discontinuation of c7E3 Fab.

Treatment Allocation

The trial was conducted in two stages. A total of 56 patients were enrolled. The purpose of the first stage was to identify a single bolus dose of c7E3 Fab that would result in a >80% reduction in the number of available GP IIb/IIIa receptor sites and a <20% platelet aggregation response to 20 μmol/L ADP at 2 hours after injection compared with baseline. Five patients each were treated with a single bolus of c7E3 Fab at 0.15, 0.20, and 0.25 mg/kg in this stage. The purpose of the second stage was to assess the effects of prolonged administration of c7E3 Fab. A total of 32 patients were given a bolus of 0.25 mg/kg c7E3 Fab immediately followed by progressively longer infusions at a rate of 10 μg/min. In this manner, 11 patients each were treated for 6 and 12 hours and 10 were treated for 24 hours. To complete the trial, 9 additional patients who otherwise matched entry criteria were given placebo and followed in the same fashion as patients treated with active drug.

Laboratory Studies

The effects of c7E3 Fab on platelet function were evaluated by serial measurements of platelet aggregation, GP IIb/IIIa integrin blockade, and bleeding time before and after drug administration. Three study sites obtained platelet aggregation and receptor blockade data, and all sites obtained bleeding time values. Blood was collected in 3.8% sodium citrate tubes. Platelet aggregation was determined by the turbidimetric method using 20 μmol/L ADP as the agonist. Assays were performed in platelet-rich plasma in either a Bio/Data PAP-4 or a Chrono-Log aggregometer. Platelet aggregation was quantified as the maximum change in light transmission occurring within 5 minutes of addition of agonist. For summary purposes, posttreatment platelet aggregation was expressed as a percentage of the individual’s pretreatment aggregation. Receptor availability was calculated by the radiometric method of Coller and Scudder. Again, receptor availability after treatment was expressed as a percentage of the individual patient’s pretreatment receptor availability. Bleeding times were determined by the Ivy template technique as performed by an automated incision-making instrument (Simplate II, Organon Teknika Corp). When bleeding continued beyond 30 minutes, the assay was terminated and the measurement was reported as a value truncated at 30 minutes.

Serum samples were also collected at baseline and serially after hospital discharge to assay for the development of human antichimeric antibodies. The antibody response was measured by use of a streptavidin-based enzyme immunoassay.
TABLE 1. Baseline Population Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=9)</th>
<th>Stage 1, Bolus C7E3 Fab (n=15)</th>
<th>Stage 2, Bolus and Infusion C7E3 Fab (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>8 (89)</td>
<td>12 (80)</td>
<td>24 (75)</td>
</tr>
<tr>
<td>Median age, y</td>
<td>56.0</td>
<td>62.0</td>
<td>57.0</td>
</tr>
<tr>
<td>Median weight, kg</td>
<td>86.3</td>
<td>84.0</td>
<td>84.6</td>
</tr>
<tr>
<td>Median platelet count, ×1000/mm</td>
<td>281</td>
<td>239</td>
<td>245</td>
</tr>
<tr>
<td>Median hemoglobin, g%</td>
<td>14.4</td>
<td>14.4</td>
<td>14.2</td>
</tr>
<tr>
<td>Median bleeding time, min</td>
<td>3.5</td>
<td>4.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Median binding sites</td>
<td>82540</td>
<td>79654</td>
<td>77983</td>
</tr>
<tr>
<td>Rest angina, n (%)</td>
<td>3 (33)</td>
<td>5 (33)</td>
<td>14 (44)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>2 (22)</td>
<td>2 (13)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Multiple lesions attempted, n (%)</td>
<td>1 (11)</td>
<td>9 (60)</td>
<td>11 (34)</td>
</tr>
</tbody>
</table>

C7E3 Fab indicates chimeric monoclonal 7E3 Fab antibody.

Clinical End Points

Clinical efficacy was determined on the basis of PTCA procedural outcome combined with absence of ischemic complications. PTCA lesion success was defined as a final visual diameter percent stenosis of <50%. A procedure was judged successful if all target lesions were reduced to a residual luminal diameter percent stenosis of <50%. Clinical efficacy was attained with a successful procedure and absence of any of the composite ischemic end points, including recurrent angina, abrupt closure, myocardial infarction, repeat urgent (or emergency) PTCA, urgent (or emergency) coronary artery bypass surgery, or death during the index hospitalization. Bleeding complications were graded by the Thrombolysis in Myocardial Infarction Study bleeding classification as major, defined as an intracranial hemorrhage or decrease in hemoglobin >5 g/dL (or 15% hematocrit); minor, defined as gross hematuria or hematemeses, decrease in hemoglobin >3 g/dL with other observed blood loss, or decrease in hemoglobin >4 g/dL without an identified bleeding site; or insignificant. Adverse clinical events were classified as being not related, possibly related, probably related, or definitely related to the administration of c7E3 Fab. All clinical events were adjudicated by an independent reviewer.

Statistical Analysis

For continuous measurements, the primary descriptive statistics were medians, means, and ranges. ANOVA was applied to assess for differences between treatment groups regarding continuous baseline characteristics as well as changes in hematologic parameters after treatment. Linear regression analysis was applied to platelet aggregation and GP IIb/IIIa integrin blockade measurements (expressed as percentages of pretreatment values) to assess for a dose-response relation. Individual data points were displayed graphically to aid in the analyses. Fisher's exact test was used to test for treatment-related differences for both recurrent ischemic events and the severity of adverse events. All statistical analyses were performed with SAS software.

Results

Baseline Patient Characteristics

Baseline characteristics are summarized in Table 1. There were no significant differences among groups in baseline clinical or angiographic variables. All patients met entry criteria and were thus judged to be at moderate to high risk of PTCA-associated ischemic complications. Multilesion PTCA was performed in 20 treated patients (43%) but in only 1 of the control subjects (11%).

Pharmacodynamic Responses

Fig 2 graphically depicts the dose responses measured at 2 hours after bolus injection of c7E3 Fab at doses of 0.15, 0.20, and 0.25 mg/kg in the stage 1
patients. Shown in this figure are dose responsiveness in terms of percentage of receptors blocked and percentage of platelet aggregation to 20 μmol/L ADP compared with baseline, with each patient serving as his or her own unique baseline. The top panel illustrates the progressive increase in GP IIb/IIIa integrin blockade that occurred with increasing bolus doses of c7E3 Fab. After a dose of 0.15 mg/kg, a median of 54% of receptors were blocked, increasing to 87% blockade at 0.25 mg/kg. Platelet aggregation in response to 20 μmol/L ADP decreased in a dose-related fashion, with a median value of 46% of baseline after a bolus dose of 0.15 mg/kg, falling to 18% at 0.25 mg/kg. Bleeding time was prolonged beyond 30 minutes at 2 hours after a 0.25-mg/kg bolus. On the basis of these findings, a dose of 0.25 mg/kg was selected for the second stage of the trial.

Kinetics of platelet functional recovery after administration of a single bolus dose of 0.25 mg/kg c7E3 Fab are illustrated in Fig. 3. Peak effects on receptor blockade, platelet aggregation, and bleeding time were observed at the first measurement time of 2 hours after bolus administration. Gradual recovery of platelet function then occurred over time. Bleeding times returned to near normal values by 12 hours.

The effects of administration of a 0.25-mg/kg bolus dose of c7E3 Fab followed by a 10-μg/min infusion for 12 hours are depicted in Fig 4. Receptor blockade, inhibition of platelet aggregation, and prolongation of bleeding time were maintained throughout the duration of the 12-hour infusion. As shown in the figure, recovery did not begin until after discontinuation of the infusion. While not illustrated, similar degrees of platelet suppression were achieved by extending the duration of infusion to 24 hours.

Clinical Outcomes

PTCA success was achieved in 67 of 70 lesions (96%) in 44 of 47 patients (94%) treated with c7E3 Fab and 9 of 10 lesions (90%) in 8 of 9 patients (89%) in the control
group. Of the 3 lesion failures in the c7E3 Fab–treated cohort, 2 lesions were not reduced despite high-pressure balloon inflations; the third was reduced only from 90% to a 70% final stenosis. None of these patients developed angiographic evidence suggestive of thrombus despite the unsuccessful angioplasty attempts.

Overall, there were no significant differences in clinical event rates among groups. Table 2 presents coronary ischemic complications from the conclusion of the PTCA to the time of discharge. No treated patients developed a thrombotic abrupt closure during or after PTCA. Of the control patients, one developed a thrombotic abrupt closure shortly after a technically successful procedure; this patient also required emergency coronary bypass surgery. One patient in the infusion treatment cohort was returned to the catheterization suite for recurrent angina. Repeat catheterization documented propagation of a major dissection from the site of the original PTCA. Repeat PTCA was not successful in repairing the dissection, and the patient required urgent coronary bypass surgery and sustained a myocardial infarction as a consequence. One additional patient in the infusion treatment cohort developed elevation of myocardial isoenzymes consistent with the diagnosis of myocardial infarction but was otherwise asymptomatic. The one death in the trial occurred in a patient with a history of interstitial lung disease and congestive heart failure whose PTCA was complicated by ventricular fibrillation. The patient died 52 days later of progressive respiratory impairment, sepsis, and multiple organ failure without developing further signs or symptoms of myocardial ischemia. This patient’s demise was thought not to be cardiac in origin or related to the administration of c7E3 Fab.

**Safety Profile**

The safety of c7E3 Fab administration was evaluated by serial measurements of hematology and coagulation parameters, assessment of bleeding complications, and documentation of transfusion requirements. No patients developed thrombocytopenia, defined as a drop in platelet counts to <50% of baseline or absolute thrombocytopenia of 80,000 cells/μL. Mild (<20%) decreases in platelet counts were observed in all groups, including control subjects, in the first 24 hours. Similarly, all groups developed a drop in mean hemoglobin concentration, ranging from a minimum mean decrease of 1.34±1.47 g/dL in patients receiving only a bolus of 0.25 mg/kg c7E3 to a maximum mean decrease of 2.32±0.98 g/dL in patients receiving only a bolus of 0.20 mg/kg of the study drug. For all of these measures, there were no significant differences among groups, nor were dose-dependent effects identified.

Of particular note is that c7E3 Fab platelet suppression could be partially reversed by the administration of fresh platelets, with recovery of normal hemostasis in two patients on transfusion of pooled random-donor platelets. A summary of bleeding events and transfusion requirements, aside from those elements attributable to coronary artery bypass surgery, is presented in Table 3.

### TABLE 3. Bleeding Events and Transfusion Requirements

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=9)</th>
<th>Stage 1, Bolus c7E3 Fab (n=15)</th>
<th>Stage 2, Bolus and Infusion c7E3 Fab (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor bleeding events, n (%)</td>
<td>0</td>
<td>4 (27)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Major bleeding events, n (%)</td>
<td>0</td>
<td>0</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Red blood cell transfusion, n (%)</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Platelet transfusion, n (%)</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Fresh frozen plasma transfusion, n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

c7E3 Fab indicates chimeric monoclonal 7E3 Fab antibody. Blood loss and blood transfusion associated with coronary bypass surgery are not listed in this table.
Minor bleeding was in large part related to oozing and hematoma formation at the vascular access sites; three patients also developed hematuria, and five manifested minor gastrointestinal bleeding. Major bleeding occurred in three treated patients. In one patient, access site oozing was severe enough to result in a drop in hemoglobin of 6.3 g/dL (thus meeting criteria for major bleeding); this patient was managed conservatively and did not receive a blood transfusion. A second patient developed severe bleeding at an access site requiring transfusion with packed red blood cells, fresh frozen plasma, and platelets immediately before urgent coronary bypass surgery. The third patient, described previously, required multiple transfusions between 8 and 21 days postinfusion for multiple organ system failure with multiple sites of bleeding (thought not to be related to study drug administration) and was counted as meeting criteria for major bleeding.

The antibody preparation was well tolerated at all dosing regimens. No patients developed clinical adverse events (other than bleeding complications) that were classified as probably or definitely related to the study agent. Four patients had the active drug terminated early because of bleeding events; none required premature termination of the drug because of a suspected side effect.

Finally, the first 25 patients in this study were serially followed for 20 weeks for the development of antibodies to c7E3 Fab. None developed serological evidence of an immune response in long-term follow-up.

Discussion

This study was the first to apply a novel antiplatelet antibody, c7E3 Fab, as a prophylactic adjunct in high-risk PTCA to attempt to reduce ischemic complications of angioplasty. Stage 1 of this trial demonstrated that c7E3 Fab produces a dose-dependent blockade of platelet GP IIb/IIIa receptors and that this receptor blockade is correlated with inhibition of platelet aggregatory function. This stage also established a bolus dose of 0.25 mg/kg c7E3 Fab for the effective suppression of platelet aggregation. Stage 2 demonstrated that inhibition of platelet function could be sustained for up to 24 hours by administration of continuous-infusion c7E3 Fab. In both stages, platelet function began to recover within 6 hours of the cessation of infusion, regardless of infusion duration.

The selection of a >80% level of GP IIb/IIIa integrin blockade with suppression of platelet aggregation to <20% of baseline as a target for pharmacological efficacy was based on the observations of several groups.12,13 In animal models of severe coronary stenosis, platelet inhibition associated with >80% receptor blockade prevented platelet thrombosis. In our study, the 0.25-mg/kg dosage produced consistent receptor blockade of >80% with potent inhibition of platelet aggregation. Furthermore, in phase 1 clinical studies, increasing the bolus dose beyond 0.30 mg/kg did not result in incremental inhibitory effects on platelet aggregation.22 Thus, this work established the 0.25-mg/kg dose as an effective bolus dose of c7E3 Fab in humans. Continuous-infusion c7E3 Fab at a rate of 10 μg/min after a 0.25-mg/kg bolus dose maintained GP IIb/IIIa integrin blockade, inhibition of ex vivo platelet aggregation, and prolongation of bleeding times at pharmaco-
logically effective levels throughout infusion intervals up to 24 hours. Previous work demonstrated that infusion of c7E3 Fab at half the rate used in this study (5 μg/min) did not maintain the same high level of GP IIb/IIIa integrin blockade and resulted in a gradual loss of platelet inhibition over the course of the infusion period.26

In addition to the observed antiaggregatory activity, the administration of c7E3 Fab in our limited study population was associated with favorable patient outcomes. Although formal statistical analyses were inappropriate in this pilot study because of the small sample size, clinical results were nonetheless better than the expected 10% to 20% ischemic complication rate that the patient risk profiles would suggest.1,2,19,20 No patient treated with c7E3 Fab developed evidence of thrombosis either during or after the PTCA procedure. Indeed, the observed procedural failures in the treated group were due to mechanical complications (failure to dilate and major dissection) unlikely to be modified by the use of a potent antiplatelet agent; more importantly, delayed ischemic complications, including the one coronary bypass procedure and one late death, were both events that occurred after PTCA and beyond the duration of action of the study drug.

The aggressive approach incorporated in this study, featuring concomitant administration of aspirin, anticoagulation with heparin, and GP IIb/IIIa integrin blockade with c7E3 Fab, was expected to increase the risk of bleeding events. A useful reference population is the group of patients with Glanzmann thrombasthenia. These patients, who have a congenital absence of a functional GP IIb/IIIa integrin, suffer from spontaneous mucocutaneous bleeding and a 1% per annum incidence of other serious bleeding.27,28 In our population, then, this baseline rate of serious bleeding would be increased because of vascular trauma associated with access to the central circulation during catheterization. Although the incidence of bleeding complications in this study was perhaps high compared with a typical angioplasty population, these complications were largely limited to the development of clinically minor oozing and hematoma formation at vascular access sites along with a modest incidence of guaiac-positive urine and feces. However, these data also suggest that the extent of use of this agent in a particular population to prevent thrombosis will depend on the balance of thrombotic and hemorrhagic risk, with applicability outside the high-risk setting predicated on improvement of the safety profile.

The administration of potent biologics, especially those with the potential for antigenicity, poses one additional potential risk that must be considered before widespread application. Previous studies of the murine 7E3 product demonstrated the potential for antigenicity manifested by the development of low titers of IgG antibodies specific for the murine antibody.15,16 The reconstructed antibody c7E3 Fab used in this study, expressly designed to minimize antigenic potential while maintaining biological activity, was well tolerated by all patients. No adverse reactions (other than bleeding) related to administration of this agent were detected, nor did any patient develop thrombocytopenia or antibodies to the active agent.
In summary, the importance of this study was twofold: first, this investigation was the first to use the potent antiplatelet antibody c7E3 Fab in patients undergoing PTCA, and second, this study provided the pharmacodynamic constructs for the appropriate administration of this new agent in larger-scale studies, particularly the EPIC Trial.\textsuperscript{17,18} The management strategy delineated herein represents a potential advance in the management of the patient at risk of thrombosis, especially in light of experimental studies suggesting that perhaps 70% of platelet deposition at the site of balloon injury is GP IIb/IIIa integrin dependent.\textsuperscript{29} Furthermore, this class of drugs may be among the first to result in a significant reduction in long-term restenosis.\textsuperscript{18,30} In fact, these were the principal findings of the EPIC Trial: suppression of platelet aggregation for a period of 12 to 18 hours at the time of coronary intervention by blockade of the GP IIb/IIIa integrin improves 30-day and 6-month measures of clinical efficacy.\textsuperscript{17,18} The main limitation of this study is the restricted scope secondary to the prespecified design goals; since this was a phase 2 preliminary investigation, the primary purpose was not to determine clinical safety and efficacy in a controlled fashion but instead to investigate the biological activity of the drug in the setting of PTCA. Although a comparison group was included in the design, these patients were not case-matched control subjects, and thus, no meaningful statistical comparisons can be performed. Nonetheless, the initial favorable procedural and clinical outcomes observed in the patients treated with c7E3 Fab support expansion of the investigation of this agent to larger randomized studies of coronary intervention and other settings of clinical thrombosis.

Appendix

Sites, Principal Investigators, and Study Coordinators


Acknowledgments

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References


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