Abciximab Suppresses the Rise in Levels of Circulating Inflammatory Markers After Percutaneous Coronary Revascularization

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Background—Previous investigators have shown that systemic markers of inflammation may be increased in patients with acute ischemic syndromes or after percutaneous coronary revascularization and that persistent elevation in these markers is predictive of excess risk of subsequent adverse cardiac events. By virtue of its cross-reactivity with the glycoprotein IIb/IIIa, αβ3, and αMβ2 receptors, abciximab may reduce inflammatory processes.

Methods and Results—Assays for the inflammatory markers C-reactive protein, interleukin-6, and tumor necrosis factor-α were performed on serum samples obtained from 160 patients in a placebo-controlled, randomized trial of abciximab during angioplasty. Eighty patients each had received a placebo or abciximab bolus plus a 12-hour infusion. Serum samples were drawn at baseline (before revascularization), 24 to 48 hours after study drug administration, and 4 weeks after study drug administration. Between baseline and 24 to 48 hours, the increase in C-reactive protein was 32% less in patients receiving abciximab than placebo (P = 0.025); the rise in interleukin-6 levels was 76% less in the abciximab group (P < 0.001); and the rise in tumor necrosis factor-α levels was 100% less with abciximab therapy (P = 0.112). By 4 weeks, most marker levels had returned to baseline, with no significant differences between placebo and abciximab groups.

Conclusions—Systemic markers of inflammation increase in the first 24 to 48 hours after angioplasty, but the magnitude of that rise is diminished by periprocedural abciximab. Some of the long-term clinical benefit derived from this agent may be related to an anti-inflammatory effect. (Circulation. 2001;104:163-167.)

Key Words: inflammation ■ angioplasty ■ platelets

The contribution of inflammation to the pathogenesis of atherosclerotic heart disease and the complications of acute ischemic syndromes and coronary revascularization has been increasingly recognized over the last several years. Elevated serum levels of acute phase proteins, which are nonspecific systemic markers of inflammation, have been associated with elevated risk for both short-term and long-term ischemic events after successful coronary angioplasty or stenting1–4 and among patients with unstable angina5–7 or myocardial infarction.8

Pharmacological agents directed against the integrin glycoprotein (GP) IIb/IIIa receptor potently inhibit platelet aggregation and thrombus formation at the injured coronary plaque.9 These agents reduce the risk of acute ischemic events by up to 50% among patients undergoing angioplasty, stenting, or atherectomy.10 In addition to the anti-thrombotic effect, however, blockade of the GP IIb/IIIa receptor may inhibit the interaction between platelets and leukocytes, and hence limit the inflammatory response to coronary intervention. Moreover, cross-reactivity of one of the GP IIb/IIIa inhibitors, abciximab (ReoPro, Centocor), with other integrin receptors, including αMβ2 (Mac-1), may have other anti-inflammatory effects.11,12

To assess whether abciximab influences the inflammatory process in patients undergoing percutaneous coronary revascularization, we measured periprocedural changes in circulating levels of systemic inflammatory markers in patients who were enrolled in the Evaluation of c7E3 for Prevention of Ischemic Complications (EPIC) randomized, placebo-controlled trial of abciximab during balloon angioplasty.

Methods

Patient Population and EPIC Trial Protocol

The patient population described in this study is a subset of 160 patients enrolled in the EPIC trial.13 In brief, 2099 patients undergoing high-risk coronary balloon angioplasty or directional atherec-
Inflammatory Markers Substudy Protocol

Blood samples were scheduled to be drawn in all patients in the EPIC trial at baseline (preintervention), hospital discharge, and 2, 4, and 12 weeks after study drug administration for measurement of the presence of human anti-chimeric antibodies. Sufficient serum was available for each patient, beyond that required for antibody testing, for aliquots to be preserved for future analyses. The patients included in this substudy were the first consecutive 160 patients enrolled (80 in each group) at 56 clinical sites throughout the United States between November 1991 and November 1992. Criteria constituting high-risk status included acute or recent myocardial infarction, unstable angina, complex target lesion angiographic morphology, or a moderately complex target lesion in association with age >65 years, female sex, or diabetes mellitus. The protocol was approved by the Institutional Review Board at each clinical site, and all patients gave informed consent.

All patients were treated with aspirin and sufficient heparin to achieve an activated clotting time 300 to 350 seconds before and throughout the coronary intervention. Patients were randomized in a double-blind fashion to 1 of 3 intravenous treatment regimens: placebo, abciximab 0.25 mg/kg bolus, or abciximab 0.25 mg/kg bolus followed by a 10 µg/min infusion for 12 hours. Coronary angioplasty or directional atherectomy were performed according to conventional techniques.

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Blood was drawn into red top Vacutainer tubes and allowed to clot at room temperature before centrifugation to separate the serum. Serum was collected and aliquoted into 2-mL Sarstedt microtubes, frozen, and shipped from the clinical sites to Centocor on dry ice. Samples were maintained at Centocor at 70°C until the inflammmatory assays were performed. Serum was analyzed for levels of high-sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) at the Mayo Central Laboratory for Clinical Trials. CRP was measured on the Hitachi 912 chemistry analyzer by a latex particle–enhanced immunoturbidimetric assay (Kamiya Biomedical Corp). Intra-assay coefficients of variation were 8.8%, 1.1%, and 0.4% at CRP concentrations of 0.028, 0.20, and 1.15 mg/dL, respectively. IL-6 was measured by a 2-site sequential chemiluminescent isometric assay on the Immulite automated immunoassay system (Diagnostic Products). Intra-assay coefficients of variation were 7.5%, 7.3%, and 4.2% at IL-6 concentrations of 12.4, 20, and 108 pg/mL, respectively. TNF-α was measured by a solid phase, 2-site chemiluminescent immunometric assay on the Immulite automated assay system. Intra-assay coefficients of variation were 12.1%, 7.0%, and 4.7% at TNF-α concentrations of 6.8, 8.5, and 282 pg/mL, respectively.

Statistical Methods
Continuous variables were summarized by means, medians, SDs, and interquartile ranges. Changes in levels of serum inflammatory markers at 24 to 48 hours and at 4 weeks were assessed relative to baseline (before study drug administration). Differences between placebo and abciximab treatment groups with respect to changes in serum markers were compared using the nonparametric Wilcoxon 2-sample test, because the data were not normally distributed. A sample size of 160 was calculated to produce an 80% power to detect a 30% difference between treatment groups with regard to the rise in inflammatory markers with a significance level of 0.05.

Results
Baseline Characteristics and Clinical Outcome
Baseline characteristics were balanced between the placebo and abciximab groups in the inflammatory markers substudy (Table 1). Patients within the substudy were largely representative of the overall EPIC trial population, although a smaller proportion of substudy patients had an acute ischemic syndrome, because of the exclusion of patients with recent myocardial infarction. The composite end point of death, myocardial infarction, or urgent revascularization by 30 days after randomization occurred in 7 patients in the placebo group (8.8%) and 1 patient in the abciximab bolus plus
Inflammatory responses have been implicated as important causative factors across the spectrum of acute and chronic ischemic events by this agent. These findings suggest that blockade of the platelet GP IIb/IIIa receptor with abciximab markedly decreases the risk of ischemic complications from percutaneous coronary revascularization, an effect which has traditionally been attributed to inhibition of platelet aggregation and thrombus formation. This current study demonstrates for the first time that abciximab also suppresses the periprocedural rise in markers of systemic inflammation. Among a subgroup of 160 patients in the placebo-controlled EPIC trial, levels of CRP, IL-6, and TNF-α increased over the 24 to 48 hours after high-risk balloon angioplasty or atherectomy. Treatment with abciximab, however, was associated with reductions of 30% to 100% in the magnitude of rise in these markers. The influence of abciximab on inflammatory markers seemed to occur independently of the inhibition of ischemic events by this agent. These findings suggest that some of the immediate or long-term benefit of abciximab in the setting of coronary intervention may be related to the suppression of inflammation.

### Discussion

Blockade of the platelet GP IIb/IIIa receptor with abciximab markedly decreases the risk of ischemic complications from percutaneous coronary revascularization, an effect which has traditionally been attributed to inhibition of platelet aggregation and thrombus formation. This current study demonstrates for the first time that abciximab also suppresses the periprocedural rise in markers of systemic inflammation. Among a subgroup of 160 patients in the placebo-controlled EPIC trial, levels of CRP, IL-6, and TNF-α increased over the 24 to 48 hours after high-risk balloon angioplasty or atherectomy. Treatment with abciximab, however, was associated with reductions of 30% to 100% in the magnitude of rise in these markers. The influence of abciximab on inflammatory markers seemed to occur independently of the inhibition of ischemic events by this agent. These findings suggest that some of the immediate or long-term benefit of abciximab in the setting of coronary intervention may be related to the suppression of inflammation.

### Inflammatory Markers

Levels of the 3 inflammatory markers before (baseline), 24 to 48 hours after, and 4 weeks after study drug administration are presented in Table 2. Changes in the levels of these markers relative to baseline are presented in the Figure. The increase in CRP level between baseline and 24 to 48 hours was 32% less in patients receiving abciximab compared with those receiving placebo ($P=0.025$), with no significant differences between treatment groups in the changes in levels out to 4 weeks. The observed rise in IL-6 levels at 24 to 48 hours was 76% less in the abciximab group than in the placebo arm ($P<0.001$), with no differences in the changes in levels at 4 weeks. The rise in TNF-α levels was 70% to 100% less in the abciximab group at both 24 to 48 hours ($P=0.112$) and 4 weeks ($P=0.051$).
phases of atherosclerotic vascular disease.\textsuperscript{14} Endothelial injury induces the expression of intercellular adhesion molecules and the release of chemoattractant compounds that mediate the recruitment, attachment, and migration of leukocytes into the arterial wall.\textsuperscript{15} Infiltrating inflammatory cells enhance oxidation and uptake of low-density lipoproteins and produce cytokines, mitogens, and reactive oxygen species, stimulating smooth muscle cell migration and proliferation and contributing to ongoing endothelial injury.\textsuperscript{16} These processes lead to the formation of the fatty streak and the mature atherosclerotic plaque. Inflammation also seems to play a role in the development of acute ischemic syndromes. Lymphocytes and monocytes accumulate at the edges of the fibrous cap, producing cytokines and matrix metalloproteinases, which enhance collagen and elastin degradation.\textsuperscript{14,17} The resultant evolution of the vulnerable plaque provides the substrate for plaque rupture, vascular thrombosis, and unstable angina or myocardial infarction. Inflammatory processes further perpetuate the thrombotic response to plaque disruption. Binding activated platelets to leukocytes facilitates thrombosis by activating factor X and providing sites for assembly of the prothrombinase complex. Platelet-leukocyte aggregates also enhance the local and systemic inflammatory state by releasing cytokines.

Markers of inflammation are consistently found to be prognostic for the prevalence of atherosclerosis, clinical manifestations of coronary artery disease, and increased risk for complications of acute ischemic syndromes or revascularization procedures. Prospective and cross-sectional studies have documented associations between levels of CRP in apparently healthy individuals and the occurrence of myocardial infarction, stroke, or cardiovascular mortality.\textsuperscript{18} Among patients with acute ischemic syndromes, elevated circulating concentrations of CRP, IL-1 receptor antagonist, or IL-6 are predictive of recurrent ischemia, myocardial infarction, and long-term mortality.\textsuperscript{5–8} Inflammatory markers have also been shown to increase in the period immediately after percutaneous revascularization procedures in patients with or without unstable myocardial ischemia, and the magnitude of rise in these markers has been correlated with subsequent myocardial infarction and restenosis.\textsuperscript{1,4–19}

The efficacy of percutaneous revascularization is considerably improved by the administration of GP IIb/IIIa receptor antagonists. In randomized placebo-controlled trials, the risk of death, myocardial infarction, or emergency repeat revascularization within 30 days after coronary intervention was reduced by \( \approx 40\% \) to \( 60\% \) with abciximab and by \( 15\% \) to \( 35\% \) with epifibatide (Integrillin, COR Therapeutics) or tirofiban (Aggrastat, Merck).\textsuperscript{10} With abciximab, clinical benefit was particularly marked among patients revascularized in the setting of unstable angina.\textsuperscript{20} Moreover, this agent has been associated with a long-term decrease in mortality, an effect that cannot be entirely attributed to the suppression of acute periprocedural ischemic events.\textsuperscript{21} Mortality reduction has not been observed to date with epifibatide or tirofiban. Apparent heterogeneity in the magnitude of treatment effect observed in these trials between the antibody fragment and the reversible small molecule inhibitors may reflect differences in the intensity and duration of receptor blockade, inadequate dosing, variations in trial design, or statistical chance. However, differences in receptor specificity among the agents may also be important. Epifibatide and tirofiban inhibit only GP IIb/IIIa, but abciximab also binds to the \( \alpha v \beta 3 \) (vitronectin) receptor on endothelial, smooth muscle, and inflammatory cells\textsuperscript{11} and to an activated conformation of the \( \alpha M \beta 2 \) receptor on leukocytes.\textsuperscript{13}

The cross-reactivity of abciximab raises the possibility that clinical benefit derived from this therapy may not be exclusively due to its antithrombotic effect, but may also be related to the suppression of inflammatory pathways involving platelets, white blood cells, and the vascular endothelium. Leukocytes initially adhere to endothelial cells via P-selectin, but firmer attachment is mediated by \( \alpha M \beta 2 \) binding, either directly\textsuperscript{22} or through fibrinogen bridging to intracellular adhesion molecule-1 on endothelial cells.\textsuperscript{23} Fibrinogen bridging of \( \alpha M \beta 2 \) and intracellular adhesion molecule-1 also enhances leukocyte migration across the endothelium.\textsuperscript{24} One of the 2 complementary mechanisms of formation of platelet-leukocyte aggregates involves fibrinogen bridging of platelet GP IIb/IIIa to leukocyte \( \alpha M \beta 2 \).\textsuperscript{25} Abciximab may not only inhibit these inflammatory processes directly by blocking GP IIb/IIIa and \( \alpha M \beta 2 \), but it also seems to reduce leukocyte surface expression of \( \alpha M \beta 2 \).\textsuperscript{26} Issues of cross-reactivity with other receptors aside, it must be emphasized that GP IIb/IIIa receptor blockade per se is sufficient to reduce platelet attachment to monocytes and endothelial cells, thus exerting an anti-inflammatory effect. Thus, the relative efficacy of the selective versus nonselective GP IIb/IIIa antagonists in reducing periprocedural inflammation can be assessed only by direct comparative studies.

In this current study, we evaluated the effect of abciximab on the rise in levels of CRP, IL-6, and TNF-\( \alpha \) after percutaneous revascularization. TNF-\( \alpha \) and IL-6 are cytokines that mediate humoral and cellular inflammatory processes in response to infection, inflammation, and tissue injury. IL-6 is expressed in a number of cell types, including those within atherosclerotic plaque, and its production seems to be controlled in part by IL-1\( \beta \) and TNF-\( \alpha \).\textsuperscript{6,19} CRP is an acute-phase reactant produced by the liver under the influence of inflammatory cytokines, principally IL-6.\textsuperscript{19} In addition to acting as a marker of a systemic inflammatory state, CRP may also have a direct pro-inflammatory effect,\textsuperscript{27} and it may influence thrombosis and inflammation through complement activation.\textsuperscript{28} Consistent with previous investigations,\textsuperscript{1–3,19} we observed an increase in serum levels of these markers, particularly CRP and IL-6, over the first 24 to 48 hours after coronary intervention. The observed suppressive effect of abciximab on the rise in IL-6 was somewhat greater than for CRP, perhaps reflecting the greater stability of CRP in the circulation (half-life of 19 hours versus 4 hours for IL-6).\textsuperscript{19} It does not seem that myocardial necrosis per se was the source of cytokine elevation in this study; patients with myocardial infarction within the prior 7 days had been excluded from consideration, and results were unchanged when the few patients experiencing postprocedural ischemic events were removed from the analysis. For the same reasons, the diminution of the postprocedural rise in inflammatory marker levels by abciximab seems to have occurred independently of
the reduction in ischemic complications. It is possible, however, that suppression of periprocedural ischemic events below the threshold of clinically detectable myocardial necrosis may have accounted for some of the effect of abciximab on inflammatory responses.

Given the relatively small number of ischemic end points that occurred in this study of 160 patients, it was not possible to detect a correlation between levels of inflammatory markers and subsequent adverse clinical events. Therefore, a cause-and-effect relationship could not be established between suppression of the rise in these markers by abciximab and the known benefits of this agent. Nevertheless, the growing body of evidence linking systemic inflammation to unfavorable short- and long-term outcome in cardiovascular disease states suggests that an anti-inflammatory effect of abciximab may have salutary clinical consequences. These data thus have implications for possible differential efficacy among various GP IIb/IIIa inhibitors and are supportive of a potential role for modifiers of the inflammatory process in reducing ischemic events in patients undergoing percutaneous coronary revascularization.

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References


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