Antiplatelet Effects of Abciximab, Tirofiban and Eptifibatide in Patients Undergoing Coronary Stenting

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OBJECTIVES
We sought to investigate whether abciximab, tirofiban and eptifibatide achieve comparable antplatelet effects with coronary stenting.

BACKGROUND
The glycoprotein (GP) IIb/IIIa antagonists abciximab, tirofiban and eptifibatide differ in chemical structure, binding site and pharmacokinetics.

METHODS
Sixty patients undergoing coronary stenting were randomly assigned to abciximab (bolus 0.25 mg/kg body weight, infusion 10 μg per min for 12 h), tirofiban (bolus 10 μg/kg, infusion 0.15 μg/kg per min for 72 h) or eptifibatide (bolus 180 μg/kg, infusion 2 μg/kg per min for 72 h). We took serial blood samples to analyze platelet function by using flow cytometry, turbidimetric aggregometry and the rapid platelet-function assay (RPFA).

RESULTS
As assessed by RPFA, platelet aggregation after 2 h of infusion was reduced to 5.9 ± 7.8% (mean ± SD) of baseline by abciximab, to 3.0 ± 5.4% by tirofiban and to 7.8 ± 7.1% by eptifibatide (p = 0.42). Turbidimetric aggregometry with adenosine diphosphate stimulation yielded similar results, whereas percent inhibition of platelet aggregation after thrombin receptor stimulation was 45.8 ± 16.8% with abciximab, 51.3 ± 17.6% with tirofiban and 52.9 ± 14.8% with eptifibatide (p = 0.37). Tirofiban and eptifibatide maintained their level of platelet inhibition during infusion. Flow cytometry revealed that the reduction in the monocyte-platelet interaction by abciximab, tirofiban and eptifibatide was not significantly different (20.0 ± 21.9%, 23.8 ± 18.2% and 21.0 ± 19.8%, respectively; p = 0.87).

CONCLUSIONS
Abciximab, tirofiban and eptifibatide, at currently recommended doses, achieved similar levels of inhibition of platelet aggregation and a similar reduction in the platelet-monocyte interaction. (J Am Coll Cardiol 2001;37:1323–8) © 2001 by the American College of Cardiology

Glycoprotein (GP) IIb/IIIa blockade reduces the risk of percutaneous coronary interventions substantially. In many countries, the GP IIb/IIIa antagonists—abciximab, tirofiban and eptifibatide—have been approved for clinical use on the basis of several phase III trials. Among these trials, however, the risk reduction by GP IIb/IIIa blockade varies.

Abciximab, tirofiban and eptifibatide differ in chemical structure, binding site and pharmacokinetics. Abciximab is the humanized chimeric Fab fragment of a monoclonal mouse antibody. Abciximab cross reacts with the αβ3 integrin on endothelial cells and smooth muscle cells and with the αMβ2 integrin (Mac-1) on myeloid leukocytes. Tirofiban-bound abciximab persists for many days.

Tirofiban is a nonpeptide tyrosine derivative that blocks arginine-glycine-aspartic binding sites of GP IIb/IIIa. The cyclic heptapeptide eptifibatide blocks the lysine-glycine-aspartic binding site. Tirofiban and eptifibatide are competitive inhibitors with short half-lives. Continuous infusion is needed for sustained platelet inhibition.

The clinical relevance of the pharmacologic differences between abciximab, tirofiban and eptifibatide is currently unclear. Thus far, there are only limited data on the differential efficacy of the three agents in the peri-interventional inhibition of platelet aggregation.

METHODS
Patient selection and study protocol. The study group consisted of patients undergoing intracoronary stent placement for symptomatic coronary artery disease. Exclusion criteria included contraindications for aspirin, ticlopidine or GP IIb/IIIa blockade, absolute indication for anticoagulation, acute myocardial infarction, serum creatinine concentration ≥2.0 mg/dl and >24 h of antecedent treatment with ticlopidine. All patients gave written, informed consent. The study was approved by our institutional Ethics Committee.

Immediately before the balloon angioplasty that preceded
stenting, the patients were assigned to one of three open-label treatment regimens by means of a computer-generated randomization scheme: 1) abciximab bolus of 0.25 mg/kg body weight, followed by a continuous infusion at 10 μg/min for 12 h (14); 2) tirofiban bolus of 10 μg/kg, followed by a continuous infusion at 0.15 μg/kg per min for 72 h (15); and 3) eptifibatide bolus of 180 μg/kg, followed by a continuous infusion at 2 μg/kg per min for 72 h (16).

The concomitant preinterventional heparin dosage was 70 U/kg in the abciximab group and 100 U/kg in the other groups. In addition, all patients received ticlopidine and aspirin (100 mg twice daily), starting 1 to 8 h before the intervention. Ticlopidine was administered twice daily at 500 mg for the first three doses, and subsequently at 250 mg for four weeks. We performed stent placement, as described previously (17).

Peripheral venous blood samples were taken before the start of drug administration, as well as 2, 3, 4, 6, 7, 24, 48, 72, and 96 h after the start of infusion. In patients randomized to tirofiban or eptifibatide, we obtained an additional sample at 2 h after discontinuation of the infusion. We collected 2.5 ml of blood in tubes with disodium ethylene diamine tetra-acetic acid for determination of platelet count by use of a Coulter Counter (Beckman Coulter, Krefeld, Germany). The details of this rapid, automated, point-of-care platelet-function test have been described previously (11,13). For analysis of platelet aggregation, we used the rapid platelet-function assay (RPFA) (Cyfix II, gift of Dr. Andreas Ruf, Karlsruhe, Germany), as described previously (11,13). Platelet-rich plasma was prepared by centrifugation at 200g for 20 min. After adjustment from baseline, 20 μmol/liter of adenosine diphosphate (ADP; Sigma, Steinheim, Germany) or 50 μmol/liter of TRAP (Sigma) was added, and aggregation was recorded for 5 min. We assessed the maximal initial slope of the increase in photovoltage as a measure of the rate of aggregation. The results are expressed as percentages of the baseline value before administration of the study drug.

Flow cytometry. Immunostaining and flow cytometry were performed without ex vivo addition of platelet agonists, as described previously (11,13). We stained leukocytes in whole blood by triple-color immunofluorescence with the use of fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies (mAbs) for the αM chain of Mac-1 (CD11b); phycoerythrin (PE)-labeled mAbs for GP Ibα of the von Willebrand factor receptor complex (CD42b); and PE-Cy5-labeled mAbs for the monocyte marker CD14 (endothoxin receptor; all mAbs from Immunotech, Hamburg, Germany). As shown earlier, binding of the anti-CD11b mAbs used in our study was not affected by administration of abciximab (11). Platelets were stained with PE-labeled CD42b mAbs and FITC-conjugated mAbs for P-selectin (CD62P, Dianova, Hamburg, Germany). Nonspecific membrane immunofluorescence is determined by use of an irrelevant isotype-matched fluorescein-conjugated immunoglobulin G (Dianova). We used a fluorescence activated cell sorter scan (Becton-Dickinson, Heidelberg, Germany) equipped with a 488-nm argon laser at 500 mW, and we analyzed 5,000 events. We identified monocytes as anti-CD14-positive events on the histogram generated by PE-Cy5 fluorescence, and platelets by size and CD42b immunofluorescence. The mean channel of fluorescence intensity was taken as a measure for antibody binding, and thus antigen surface exposure.

Statistical analysis. The primary end point was the inhibition of platelet aggregation, as assessed by RPFA after 2 h of infusion. We considered abciximab as the reference drug, because abciximab was the only drug with documented clinical efficacy in the setting of stenting (14). We intended an 80% power to detect a 10% difference in inhibition of platelet aggregation between abciximab and one of the two other agents, with a level of significance (p value) <0.05. On the basis of earlier studies, we assumed a standard deviation of 10% for variables of platelet aggregation (13). Thus, we obtained a sample size of 20 patients in each group.

Discrete variables, expressed as counts, were tested by using the Fisher exact test. The results of continuous variables were reported as the mean value ± SD, and were...
tested by analysis of variance, followed by the Scheffé test, where appropriate. To account for potential deviations from normal distribution, we corroborated these analyses by nonparametric testing using the Kruskal-Wallis test, followed by the Mann-Whitney U test, where appropriate. Unless stated otherwise, the results of the nonparametric tests confirmed those of the parametric tests. A p value <0.05 (two-tailed test) was regarded as significant.

RESULTS

Study cohort, platelet counts and clinical events. The study groups were not significantly different with respect to major demographic, angiographic and procedural variables (Table 1). In all patients, stenting achieved residual stenosis <15%, with Thrombolysis In Myocardial Infarction (TIMI) flow grade 3. Platelet counts at baseline (Table 1) and during follow-up were not significantly different between the three groups, and we did not find a significant decrease in the average platelet count after the intervention in any of the groups. Nevertheless, in one patient assigned to abciximab, the platelet count dropped to 16 \(10^3/\mu l\) at 24 h after infusion (confirmed with a citrated sample); otherwise, thrombocytopenia \(\leq 50 \, 10^3/\mu l\) was not encountered in any of the patients. Access site complications requiring surgical intervention, or serious bleeding complications, such as hemorrhagic stroke, gastrointestinal or other bleeding necessitating transfusion, did not occur in either group. During 30-day follow-up, none of the patients incurred a serious adverse cardiac event, including death, large myocardial infarction (Q-wave or creatinine kinase \(>5\) times the upper limit) or target vessel repeat intervention.

Inhibition of platelet aggregation. As assessed by RPFA (Fig. 1), all agents achieved a mean inhibition of platelet aggregation >80% during infusion. This mean level of inhibition was maintained during infusion of tirofiban and eptifibatide. After discontinuation of infusion, recovery of platelet aggregation was delayed with abciximab and more rapid with tirofiban and eptifibatide (Fig. 1). With abciximab and tirofiban or eptifibatide, there were no significant differences in either mean platelet inhibition or the percentage of patients achieving >80% inhibition at 2 h (Fig. 2).

The time courses of the mean rate of ADP-induced platelet aggregation (Fig. 3), as assessed by turbidimetric aggregometry, were largely similar to those of RPFA. When comparing the effects of abciximab at 2 h with those of tirofiban or eptifibatide (Fig. 2), we did not find significant differences in the mean inhibition or the percentage of patients achieving >80% inhibition at 2 h (Fig. 2).
than inhibition of ADP-induced platelet aggregation (Fig. 4). At 2 h, the rate of TRAP-induced platelet aggregation was inhibited by only ~50% on average, but there were no significant differences between the three agents (p = 0.37).

Inhibition of the platelet-monocyte interaction and P-selectin exposure. With all three agents, GP Ibα fluorescence of the monocyte population decreased significantly (p < 0.005) after 2 h and 24 h of infusion (Fig. 5, left panel). This decrease was caused by a reduction in GP Ibα fluorescence intensity of the GP Ibα-positive monocytes, whereas the percentage of GP Ibα-positive monocytes remained essentially constant (data not shown). Among the three agents, there were no significant differences in the reduction of GP Ibα fluorescence on monocytes (Table 2). The reduction in the platelet-monocyte interaction by GP IIb/IIIa antagonists was associated with a reduction in surface expression of Mac-1 on GP Ibα-positive monocytes (Fig. 5, right panel). Again, this effect was not significantly different between the three agents (Table 2).

With all three agents, we found a significant (p < 0.041) increase in the percentage of P-selectin-positive platelets, maximal at 2 h. This increase in P-selectin-positive platelets did not show any significant differences between the three agents (Table 2).

DISCUSSION

Our prospective, randomized trial investigated the platelet effects of three approved GP IIb/IIIa antagonists at currently recommended dosages in the setting of coronary stenting. Our major findings are: among abciximab, tirofiban and eptifibatide, there are no significant differences in: 1) the mean inhibition of the rate of platelet aggregation induced by ADP or thrombin-receptor stimulation at 2 h of infusion; 2) the mean inhibition of platelet-monocyte interactions; and 3) mean alpha-degranulation, as a measure of antagonist-induced platelet activation.

Platelet aggregation. As in previous studies, analysis of whole blood after low dose thrombin receptor stimulation by the automated RPFA yielded results similar to those of analysis of platelet-rich plasma after stimulation with high
dose ADP by turbidimetric aggregometry (20,21). Both methods consistently showed a mean inhibition of platelet aggregation >90% at 2 h, with no significant differences between the three agents. With stimulation by high dose TRAP, the mean inhibition of platelet aggregation by the three agents was substantially weaker, albeit not significantly different among them. In an earlier study (22), abciximab inhibited thrombin-induced platelet aggregation only by ~50%, despite ~80% inhibition of ADP-induced platelet aggregation. This discrepancy can be explained by mobilization of nonblocked GP IIb/IIIa receptors by the most potent platelet stimulus thrombin (22,23). Our study demonstrates that this mechanism affects the antiplatelet efficacy of tirofiban and eptifibatide to a similar extent.

Although the optimal level of platelet inhibition at various time points has not been definitely established (24–27), it is commonly accepted that >80% inhibition of ADP-induced platelet aggregation is needed for effective prevention of thrombotic events after coronary interventions. We therefore analyzed those patients achieving this level of inhibition, and again, we found no significant differences between the three agents. It has to be noted, however, that while most patients achieved >80% inhibition, 8.3% did not by RIPA and 10% did not by turbidimetric aggregometry. Hence, there is still room for improved dosing approaches, such as the double bolus regimen recently pursued in the Enhanced Suppression of the Platelet IIb/IIIa Receptor with Integrilin Therapy (ESPRIT) trial (28).

Platelet-monocyte interaction. We addressed heterotypic adhesion of platelets to leukocytes, which appears to play a role in the regulation of inflammatory responses in ischemic heart disease (11,29,30). Recently, we showed that abciximab reduced the mass of platelets attached to circulating myeloid leukocytes in patients with acute myocardial infarction (11). The reduction in the platelet-monocyte interaction by abciximab was associated with downregulation of surface expression of Mac-1, one of the major leukocyte adhesion molecules (11). Xiao et al. (31) described a reduction of the platelet-neutrophil interaction by tirofiban, but they did not address the consequential changes in Mac-1 expression. In this study, we show that inhibition of the platelet-monocyte interaction by abciximab, tirofiban and eptifibatide is similar and leads to a comparable down-regulation of Mac-1 in circulating platelet-leukocyte aggregates.

Agonist-induced platelet activation. We found a comparable increase in P-selectin surface expression after abciximab, tirofiban and eptifibatide. Engagement of the GP IIb/IIIa receptor by natural ligands or antagonists induces platelet activation (12,32,33). Recently, we were able to demonstrate antagonist-induced platelet activation in patients treated with abciximab, by showing an abciximab-induced increased P-selectin surface expression (13). By demonstrating a similar increase in P-selectin surface expression with all three agents, our current study does not suggest that there are major differences in antagonist-induced platelet activation in the clinical setting.

Study limitations. Our study was not powered to specifically address the proportions of patients reaching a certain level of platelet inhibition. Nevertheless, the distribution of data points (Fig. 2) does not suggest major differences.

We intended to analyze the three GP IIb/IIIa inhibitors at currently recommended dosages, as well as concomitant medications. Therefore, heparin dosing differed between the three treatment arms. We cannot fully exclude that this might have had an effect on our platelet function studies. Heparin can directly activate platelets, and yet may limit thrombin-induced platelet activation.

Our study focused on platelet effects. Contrary to tirofiban and eptifibatide, abciximab has nonplatelet effects, such as inhibition of the vitronectin receptor and Mac-1. In this respect, it is noteworthy that with abciximab, but not with the other agents, downregulation of the number of surface-expressed Mac-1 complexes in platelet-leukocytes aggregates is complemented by direct Mac-1 blockade (6). Such nonplatelet effects were not addressed in our study (6,7).

Implications. By demonstrating comparable platelet effects of various GP IIb/IIIa inhibitors, the data reported here yielded a rationale for trials that directly compare the clinical efficacy of various GP IIb/IIIa inhibitors, such as TARGET (do Tirofiban And ReoPro Give similar Efficacy outcomes Trial) (34). In the meantime, the 30-day primary end point analysis of TARGET was reported. It showed a significantly better outcome with abciximab than with tirofiban. Among potential explanations for the superiority of abciximab in TARGET, our study points toward the Mac-1 and vitronectin receptor-dependent nonplatelet effects, and it

Table 2. Relative Changes in Platelet-Monocyte Interaction, Mac-1 Expression on Monocytes With Adherent Platelets and P-Selectin Surface Expression on Platelets

<table>
<thead>
<tr>
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<th>Abciximab</th>
<th>Tirofiban</th>
<th>Eptifibatide</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔGP Ib fluorescence on monocytes</td>
<td>−20.0%</td>
<td>−23.8%</td>
<td>−21.0%</td>
<td>0.87</td>
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<tr>
<td>ΔMac-1 fluorescence on GP Ib-positive monocytes</td>
<td>−27.1%</td>
<td>−24.7%</td>
<td>−23.7%</td>
<td>0.93</td>
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<tr>
<td>ΔP-selectin-positive platelets</td>
<td>53.6%</td>
<td>44.0%</td>
<td>67.20%</td>
<td>0.76</td>
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Data are presented as the mean value (95% confidence interval). Confidence intervals that do not include 0 indicate significant changes at p < 0.05.

GP = glycoprotein.
refutes failure of platelet inhibition during the steady state of infusion at 2 h and beyond.

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REFERENCES


