Platelet Glycoprotein IIb/IIIa Receptor Blockade Improves Vascular Nitric Oxide Bioavailability in Patients With Coronary Artery Disease

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Background—Platelet glycoprotein IIb/IIIa receptor blockade not only enhances epicardial flow but also improves microvascular perfusion. Inhibition of abnormal platelet–endothelial interactions may contribute to this beneficial effect. The present study was designed to determine whether glycoprotein IIb/IIIa receptor blockade influences endothelial vasomotor function and NO bioactivity in patients with coronary artery disease.

Methods and Results—Forty patients with symptomatic coronary artery stenosis were studied before planned percutaneous coronary intervention. By using venous occlusion plethysmography, endothelium-dependent and -independent vasodilation was determined by measuring forearm blood flow responses to acetylcholine with and without N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) and sodium nitroprusside. Vascular function tests were repeated during glycoprotein IIb/IIIa receptor blockade by tirofiban in 27 patients and by eptifibatide in 13 patients. A subgroup of 10 patients was retested 6 hours after stopping infusion of tirofiban. Glycoprotein IIb/IIIa receptor blockade by both substances improved acetylcholine-induced vasodilation and L-NMMA responses. Six hours after withdrawal of tirofiban infusion, the beneficial effects were not evident. Sodium nitroprusside–induced vasodilation was not changed by glycoprotein IIb/IIIa receptor blockade.

Conclusions—These findings support the concept that abnormal platelet-endothelial interactions contribute to endothelial dysfunction and impaired NO bioactivity in patients with symptomatic coronary artery disease. (Circulation. 2003;108:536-541.)

Key Words: coronary artery disease ▪ platelets ▪ endothelial dysfunction ▪ nitric oxide
tomatic coronary artery disease. In addition, to assess any effect on NO activity, N^o^-monomethyl-L-arginine (L-NMMA)–induced vasoconstriction was assessed before and during glycoprotein IIb/IIIa receptor blockade.

Methods

Patient Population

Patients who had been referred for PCI of symptomatic coronary artery disease were eligible for the study. The inclusion criteria were angiographically documented critical stenosis for which concomitant use of glycoprotein IIb/IIIa receptor blockade was planned and a stable condition. Forty consecutive patients were included in the study. Exclusion criteria included acute myocardial infarction; evidence of heart failure; uncontrolled hypertension; bleeding abnormalities; and/or significant endocrine, hepatic, renal, or inflammatory disease. Vasoactive medications—including calcium-channel blockers, β-blockers, ACE inhibitors, and long-acting nitrates—were withheld for ≥18 hours before the study. All patients were taking aspirin on a daily dose of 100 mg/day. No patients received prior thienopyridines or glycoprotein IIb/IIIa inhibitors for at least 60 days. The study was approved by the local ethics committee, and informed consent was obtained from all participants.

Vascular Function

Vascular function tests were performed after a 12-hour overnight fast and before planned PCI. With the use of sterile conditions and 2% lidocaine, a 20-gauge polyethylene catheter was inserted into the brachial artery of the nondominant arm for measurement of blood pressure and infusion of drugs. Forearm blood flow (FBF) was measured by venous occlusion plethysmography with calibrated mercury-in-silastic strain gauges as previously described. During FBF measurement, circulation to the hand was excluded by a wrist cuff, inflated 40 mm Hg above systolic blood pressure. At the beginning of each study protocol, normal saline (0.9% sodium chloride) was infused intra-arterially at a rate of 0.4 mL/min. Endothelium-dependent vasodilation was assessed by infusing acetylcholine (ACh) in increasing concentrations of 7.5, 15, and 30 μg/min into the brachial artery. Sodium nitroprusside (SNP) was infused to assess endothelium-independent vasodilation (1, 3, and 10 μg/min). The sequence of ACh and SNP infusion was randomized. Then, during confusion of NO synthase inhibitor L-NMMA (16 μmol/min), the dose-response curve to ACh was repeated.

Study Protocol

The study consisted of 3 different protocols to evaluate the following objectives: (1) effect of glycoprotein IIb/IIIa receptor blockade with tirofiban on ACh, SNP, and L-NMMA responses; (2) on-off effect of tirofiban by re-evaluating a subgroup of patients 6 hours after withdrawal of tirofiban; and (3) effect of glycoprotein IIb/IIIa receptor blockade with eptifibatide on ACh, SNP, and L-NMMA responses.

First, all patients received a baseline evaluation of vascular function during infusion of saline, ACh, SNP, and L-NMMA at increasing doses. Thereafter, in the first protocol, patients assigned to treatment with tirofiban received an intravenous bolus of 10 μg/kg body weight, followed by continuous intravenous infusion of 0.15 μg · kg^-1 · min^-1. After 30 minutes, vascular function tests with ACh, L-NMMA, and SNP were repeated during continuous tirofiban administration.

The second protocol was performed in a subgroup of patients assigned to tirofiban. After evaluation of vascular function during glycoprotein IIb/IIIa receptor blockade (tirofiban on-effect), tirofiban infusion was stopped, and additional assessment of vascular function was performed 6 hours after withdrawal of tirofiban (tirofiban off-effect).

In the third protocol, patients assigned to treatment with eptifibatide received an intravenous bolus of 180 μg/kg body weight, followed by continuous intravenous infusion of 2.0 μg · kg^-1 · min^-1. After 30 minutes, vascular function tests with ACh, L-NMMA, and SNP were repeated during continuous eptifibatide infusion. After completion of vascular function tests, all patients went for planned coronary intervention.

We also measured plasma cholinesterase activity before and during glycoprotein IIb/IIIa receptor blockade by using a cholinesterase activity assay (enzymatic activity-kinetic test, Roche) to address a potentially confounding influence of cholinesterase to our results.

Rapid Platelet Function Assay

Platelet function was determined at the following 3 time points: baseline (immediately before the glycoprotein IIb/IIIa antagonist bolus), 30 minutes after bolus (during the infusion), and 6 hours after infusion stop. Blood was collected from the indwelling brachial arterial sheath in blood tubes containing Phe-Pro-Arg chloromethyl ketone (PPACK) as the anticoagulant. For analysis of platelet aggregation, the rapid platelet-function assay (Ultegra RFPA-TRAP, Accuremetrics products, Radiometer GmbH) was used as previously described. We report RFPA data as percentages of the baseline value before administration of the study drug.

Statistical Analysis

The primary end point of the study was the effect of treatment with tirofiban on ACh responses, and we calculated the sample size for a power of 80% and a significance level of 0.05. Responses to L-NMMA were secondary end points. All values are reported as mean±SEM or number (percentage). Group comparisons with respect to baseline characteristics were performed by unpaired t test. Responses to ACh, SNP, and L-NMMA with and without glycoprotein IIb/IIIa receptor blockade are presented as mean±SEM and were analyzed by ANOVA for repeated measures, and the Scheffé test was applied for multiple comparison testing. A value of P<0.05 was considered statistically significant.

Results

Patient Characteristics

The study group consisted of 40 patients with documented coronary artery disease. All patients had critical and symptomatic coronary stenosis for planned PCI. Twenty-seven patients were randomized to receive treatment with tirofiban (group A), and 13 patients received treatment with eptifibatide (group B). Baseline characteristics of the 2 groups are presented in the Table. All subjects included in the present study were male. Both groups were comparable in terms of age, lipid profile, smoking habit, and previous myocardial infarction. Cholinesterase activity was measured before and
During inhibition of platelet aggregation with tirofiban, FBF increased to 6.5 mL·100 g/min·min−1, which was significantly larger than during saline infusion. However, the L-NMMA effect was significantly larger during inhibition of platelet aggregation with tirofiban. ACh-induced vasodilation was significantly increased to 14.7 mL·100 g/min·min−1 during saline and during tirofiban. *P<0.01 vs L-NMMA+tirofiban.

**Effect of Tirofiban on Peripheral Vasoreactivity**

During saline infusion, intra-arterial administration of ACh increased FBF of group A from 2.8±0.1 mL·100 g/min·min−1 to maximally 9.7±1.1 mL·100 g/min·min−1. During inhibition of platelet aggregation with tirofiban, ACh-induced vasodilation was significantly increased to maximally 14.7±1.3 mL·100 g/min·min−1·μg−1 (P<0.001 by ANOVA) (Figure 1A). Under saline infusion, coadministration of the NO synthase inhibitor L-NMMA blunted the ACh-induced vasodilation to a small amount of maximally 3.7±0.1 mL·100 g/min·min−1·μg−1. During tirofiban infusion, however, the L-NMMA–induced inhibition of ACh response was markedly increased (Figure 1B).

In contrast, endothelium-independent vasodilation to SNP was not modified by tirofiban. Under saline infusion, FBF increased to 6.7±0.3, 9.6±0.7, and 14.7±1.1 mL·100 g/min·min−1·μg−1 in response to SNP 1, 3, and 10 μg/min, respectively. During inhibition of platelet aggregation with tirofiban, FBF increased to 6.5±0.4, 9.4±0.8, and 14.3±1.1 mL·100 g/min·min−1·μg−1 in response to SNP 1, 3, and 10 μg/min. Six hours after tirofiban infusion stop, a subgroup of 10 patients was retested for platelet aggregation and peripheral vasoreactivity (Figure 2). During inhibition of platelet aggregation by tirofiban, residual platelet aggregation was 18±8% of baseline. After 6 hours without tirofiban infusion, residual platelet aggregation reached 76±7% of baseline (Figure 2A). ACh-induced vasodilation was significantly improved during tirofiban. Six hours after withdrawal of tirofiban infusion, however, ACh response was not different from baseline (Figure 2B). Endothelium-independent vasodilation to SNP was not changed during tirofiban infusion and 6 hours after withdrawal of tirofiban compared with baseline evaluation (data not shown).

**Effect of Eptifibatide on Peripheral Vasoreactivity**

Administration of eptifibatide increased ACh-induced vasodilation of group B significantly. The maximal FBF response averaged 10.6±1.1 mL·100 g/min·min−1·μg−1 during saline and reached 15.1±1.2 mL·100 g/min·min−1·μg−1 in response to eptifibatide treatment (Figure 3A). Coinfusion of the NO synthase inhibitor L-NMMA reduced the ACh-induced increases in FBF, being significantly greater after eptifibatide administration (ACh, 7.5, 15, and 30 μg/min; saline+L-NMMA, 1.1±0.1, 2.2±0.5, and 4.8±0.9 mL·100 g/min·min−1·μg−1; and eptifibatide+L-NMMA, 2.4±0.2, 4.4±0.9, and 8.6±1.1 mL·100 g/min·min−1·μg−1; P<0.01). Endothelium-independent vasodilation to SNP was not modified by eptifibatide (Figure 3B).
Of note, several human studies have demonstrated that endothelial damage or dysfunction would markedly shift the balance of vasoactive compounds toward vasoconstriction. Endothelial damage or dysfunction would markedly shift the balance of vasoactive compounds toward vasoconstriction. Hence, endothelial dysfunction or impairment of NO synthase inhibitor L-NMMA was significantly increased during platelet glycoprotein IIb/IIIa blockade, indicating that platelet glycoprotein IIb/IIIa blockade can markedly improve endothelium-dependent vasodilation in patients with symptomatic coronary artery disease.

Figure 3. Effect of platelet glycoprotein IIb/IIIa blockade with epifibatide on ACh-induced and SNP-induced increase in FBF. Epifibatide improved ACh-induced vasodilation significantly (A, P<0.01), but had no effect on SNP-induced vasodilation (B, P=NS).

Discussion

The present study demonstrates that platelet glycoprotein IIb/IIIa receptor blockade can markedly improve endothelium-dependent vasodilation in patients with symptomatic coronary artery disease. The inhibitory effect of the NO synthase inhibitor L-NMMA was significantly increased during platelet glycoprotein IIb/IIIa blockade, indicating that the beneficial effect of glycoprotein IIb/IIIa blockade is mainly owing to enhanced NO bioactivity. These findings point to an important role of platelet–endothelial interactions contributing to endothelial dysfunction and impaired NO bioactivity in symptomatic coronary artery disease.

There are several lines of evidence that platelet–vessel wall interactions are decisively modulated by the functional integrity of the endothelial cell layer. In the presence of an intact endothelium, activated platelets cause relaxation of isolated human arteries owing to the release of ADP and ATP. In contrast, arteries devoid of endothelial cells or with dysfunctional endothelium markedly contract to aggregating platelets, a phenomenon that is supposed to be mediated by platelet-derived thromboxane A2, serotonin, or an excessive amount of oxygen-derived free radicals. Because vascular endothelium plays an important role in the defense against vasoconstrictor substances released from activated platelets, endothelial damage or dysfunction would markedly shift the balance of vasoactive compounds toward vasoconstriction. Of note, several human studies have demonstrated that atherosclerosis and its risk factors are accompanied by abnormal endothelial vasodilator function and are associated with a defect in the platelet inhibitory effects of the vascular endothelium. Similarly, patients of the present study showed marked impairment of endothelium-dependent vasodilation to ACh and attenuated effect of the NO synthase inhibitor L-NMMA, implying reduced bioactivity of NO. Therefore, this impaired endothelial NO activity may predispose these patients to abnormal platelet–vessel wall interaction with increased vasoconstrictor effects of platelet-derived substances.

Platelet activation is a consistent finding in patients with symptomatic coronary artery disease and can persist despite aspirin therapy. Increased platelet aggregability and decreased platelet responsiveness to antiaggregatory effects of NO donors have been demonstrated in these patients. The precise mechanism underlying abnormal platelet function is not known, but increased oxidant stress has been put forward as a putative biochemical link. In addition, aggregating platelets from patients with acute coronary syndromes produce less NO compared with those from patients with inactive coronary disease. Because platelets themselves are a rich source of reactive oxygen species (ROS), abnormal platelet activation could be both a cause and a consequence of excessive oxidant stress. Altered platelet function owing to abnormal intraplatelet redox state has been proposed by several groups, demonstrating that intracellular radical scavenger could restore redox state and normal platelet function. Of note, vasodilator responses to these altered platelets are profoundly impaired, and vasoconstrictor responses are augmented compared with platelets from normal controls. Thus, alterations of platelet function may contribute to abnormal vascular responses and impaired microvascular perfusion in patients with symptomatic coronary artery disease.

Platelet inhibition by glycoprotein IIb/IIIa blockade has been shown to have beneficial effects on vascular function in various studies. In vitro experiments with isolated rings of rat aorta demonstrated that platelet-induced vasoconstriction in de-endothelialized vessels is inhibited by platelet glycoprotein IIb/IIIa blockade. Another study showed that during progressive coronary arteriostenosis in swine, treatment with a glycoprotein IIb/IIIa receptor inhibitor was able to maintain the distal coronary vasodilatory response, whereas heparin and aspirin had no effect. The investigators suggest that inhibition of platelet glycoprotein IIb/IIIa receptors may prevent the release of some vasoconstrictive substances and, as a result, help to preserve the distal coronary vasodilatory reserve. Furthermore, beneficial effects of glycoprotein IIb/IIIa receptor blockade on vasomotor function have also been found in a rabbit model of endotoxin-induced endothelial dysfunction. In this study, treatment with glycoprotein IIb/IIIa blockade could restore endothelium-dependent relaxation to ACh, but had no effect on endothelium-independent vasodila-
lation to SNP. This finding strongly suggests that glycoprotein IIb/IIIa blockade protects against dysfunctional platelet-endothelial interactions rather than a nonspecific interaction with the vascular wall. This concept is further supported by a recent study showing that glycoprotein IIb/IIIa blockade could selectively attenuate microvascular endothelial dysfunction after coronary stenting. Of note, the improved ACh response in the present study is mainly attributable to increased bioactivity of NO, because the L-NMMA effect was markedly improved during glycoprotein IIb/IIIa blockade. This beneficial effect was not evident when treatment with tirofiban was stopped for a period of 6 hours, clearly indicating that improvement of endothelial function was secondary to platelet inhibition. In addition, similar beneficial effects on endothelial dysfunction were observed in response to epifibatide, demonstrating that the effects seen with tirofiban represent a class effect of glycoprotein IIb/IIIa inhibitors rather than a specific effect restricted to the substance tirofiban.

The precise mechanisms underlying glycoprotein IIb/IIIa blockade–induced improvement of vascular NO bioactivity remain unclear. As discussed, activated platelets themselves are rich sources of ROS, including superoxide. It is therefore tempting to speculate that ROS released in response to platelet homotypic and/or heterotypic interactions may decrease vascular NO bioavailability, leading to impaired vasodilator responses and therefore to endothelial dysfunction. Furthermore, the formation of heterotypic aggregates between platelets and leukocytes and their inhibition by glycoprotein IIb/IIIa blockade may represent an important mechanism, especially as the leukocytes in these aggregates are also activated and release abundant ROS. In addition, stimulated platelets have been shown to be rich sources of chemokines and cytokines, and they serve as a reservoir for proinflammatory activity. Stimulated platelets can also directly initiate an inflammatory response on endothelial cells. Interestingly, recent experimental studies demonstrated that inflammation profoundly impairs endothelium-dependent vasodilation in human microvascular circulation, which can be prevented by pretreatment with a high dose of aspirin. One of the most important inflammatory platelet mediators is the CD40 ligand (CD40L), and increased levels of circulating soluble CD40L have been found in patients with unstable angina. Of note, it has been recently shown that CD40L reduces endothelial NO bioavailability by increasing endothelial production of ROS. Because glycoprotein IIb/IIIa receptor antagonists have an inhibitory action on the release and effects of CD40L, this may represent another important mechanism whereby glycoprotein IIb/IIIa inhibition exerts beneficial effects on endothelial function and endothelial NO bioactivity.

Apart from inhibition of homotypic and heterotypic platelet interactions, glycoprotein IIb/IIIa receptor blockers have been shown to have intrinsic activating properties. Because platelets are also an important source of NO, enhanced platelet NO production after binding of glycoprotein IIb/IIIa receptor blockers has to be considered as a potential mechanism for the observed effects.

In conclusion, the present study demonstrates that platelet glycoprotein IIb/IIIa receptor blockade improves endothelium-dependent and NO-mediated vasodilation in the human forearm of patients with symptomatic coronary artery disease. These findings clearly suggest that platelet activation per se may represent not only a marker but also an important mediator of endothelial dysfunction.

Acknowledgment

This study was supported by a grant from MSD Sharp and Dohme GmbH, Germany.

References


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Circulation. 2003;108:536-541; originally published online July 21, 2003;
doi: 10.1161/01.CIR.0000081774.31064.62

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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