Cerebral Microembolism Is Blocked by Tirofiban, a Selective Nonpeptide Platelet Glycoprotein IIb/IIIa Receptor Antagonist

Ulrich Junghans, MD; Mario Siebler, MD

Background—Microembolic signals (MES) as detected by transcranial Doppler ultrasound define an individual stroke risk in patients with carotid artery disease. To study the composition of MES in vivo, we used the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor antagonist tirofiban, a highly selective platelet aggregation inhibitor.

Methods and Results—Twenty-four patients with recent cerebral or retinal embolism of arterial origin and a MES rate >6 per hour on initial transcranial Doppler ultrasonography recording received the short-acting GPIIb/IIIa antagonist tirofiban. With tirofiban, the MES rate dropped from a median (range) of 38 (9 to 324) to zero in all patients. After cessation of infusion, the inhibitory effect of tirofiban was reversible, with a significant increase of MES (median 13.5; range, 0 to 35; n=16; P=0.001). Six patients received overlapping oral antiplatelet agents and remained MES-negative.

Conclusions—Cerebral microembolism of arterial origin has the property of solid emboli, with platelet-fibrinogen units as predominant constituent parts. GPIIb/IIIa antagonists may have the potential to bridge the ischemic risk in patients with unstable carotid disease. (Circulation. 2003;107:2717-2721.)

Key Words: atherosclerosis • embolism • receptors • stroke • platelets

Circulating cerebral microemboli (MES) can be detected by transcranial Doppler ultrasonography (TCD) in a considerable proportion of patients with recent cerebral or retinal ischemia.1–2 The clinical significance of these embolic signals is supported by the finding that their presence independently predicts early ischemic recurrence in patients with stroke and transient ischemic attacks of presumed arterial origin.3–6 Circumstantial evidence suggests that cerebral microemboli originate from the ulcerated arterial plaque.7–11 Activated platelets play a crucial role in the generation of embolic events. Irrespective of the activating stimulus, the resulting platelet aggregation is mediated by glycoprotein IIb/IIIa (GPIIb/IIIa) receptors.12 These fibrinogen receptors are members of the integrin family and exclusively expressed on platelets. Activated GPIIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets, thereby facilitating subsequent platelet aggregation and accumulation.13 The intravenously administered nonpeptide GPIIb/IIIa-receptor antagonist tirofiban specifically inhibits fibrinogen-dependent platelet aggregation in a highly selective and efficient manner with minutes after bolus application. In contrast to common antiplatelet agents such as aspirin or thienopyridines, the blocking effect of tirofiban is independent from the respective platelet-activating mechanism. Because of the short elimination half-life of tirofiban (t1/2 ≈ 2 hours), platelet function fully recovers within hours after cessation of infusion.14 In this instance, we used tirofiban in this study to examine the hypothesis that cerebral microembolic signals of arterial origin in vivo are composed of platelet-fibrinogen units as constituent parts.

Methods

Twenty-four symptomatic patients (either with recent transient ischemic attack, amaurosis fugax, or ischemic stroke) with active embolization (>6 MES per hour on initial TCD recording) were recruited. The study was approved by the local ethics committee, and all patients gave informed consent. None of the patients had potential sources of cardiogenic embolism, that is, atrial fibrillation and prosthetic heart valves were absent in all these patients. At baseline, cerebral hemorrhage was ruled out in all cases by computed tomography or MRI.15 Standard diagnostic workup used to define the respective embolic source included continuous wave Doppler sonography, color Doppler-assisted duplex imaging, transcranial Doppler sonography, intracranial magnetic resonance angiography, transthoracic and/or transesophageal echocardiography, and in some cases, digital subtraction angiography.

The study consisted of two phases: an open-label tirofiban infusion phase I and a tirofiban postinfusion phase II (Figure 1). During phase I, all patients received body weight–adjusted intravenous tirofiban with a bolus dose of 0.4 μg/kg body wt per minute for 30 minutes followed by a continuous infusion of 0.1 μg/kg body wt per minute, together with intravenously administered unfractionated heparin (UFH) targeted to an activated partial thromboplastin time (aPTT) of 50 to 70 seconds, according to the Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) protocol.16 Blood
tests to monitor platelet and red blood cell counts as well as hemoglobin, hematocrit, and aPTT were carried out within 6 hours after initiation of tirofiban infusion. These laboratory markers were monitored at least once per day until 2 days after cessation of tirofiban infusion.15

Cerebral microemboli detection was performed for a duration of 20 to 30 minutes at 3 intervals: before initiation of tirofiban infusion (iTCD), within 24 hours after initiation of tirofiban infusion, that is, at the end of phase I (first follow-up, TCD-I), and at least 2 hours after cessation of tirofiban infusion, that is, at the end of phase II (second follow-up, TCD-II). After TCD-I, patients were either continued on UFH (group A) or were given overlapping antiplatelet agents (group B), as decided by the treating physician not involved in the study.

TCD recordings were made with the use of an EME/Pioneer 4040 equipped with a 2-MHz pulsed-wave transducer. In all but one case, we insonated the middle cerebral artery ipsilateral to the qualifying source and revealed arterial embolic origin in all patients (Table). Extracranial carotid artery disease was the leading cause (n=20) of this finding. In those patients who had ischemic stroke the median time between symptom onset and tirofiban treatment was 3.2 (range, 0.15 to 38) days and the median infarct age at the end of tirofiban infusion was 6.7 (range, 2 to 39) days. None of the study patients had symptomatic cerebral or extracerebral hemorrhagic complications.

At the time of iTCD examination, 25% of patients had received neither anticoagulants nor oral antiplatelet agents; whereas 67% were already receiving treatment with UFH, 42% had oral antiplatelet agents (Table). The median MES rate at iTCD was 38 (range, 9 to 324) per hour. On follow-up TCD-I, performed within 24 hours (median, 13.5; range, 2.3 to 22 hours) after initiation of tirofiban infusion, the MES rate dropped significantly to zero in all 24 patients (P<0.0001). After TCD-I, 18 patients continued to received UFH alone after cessation of tirofiban infusion (group A, Figure 1), with 2 patients lost to follow-up due to carotid endarterectomy (CEA) before TCD-II. The remaining 6 patients received overlapping antiplatelet agents before tirofiban infusion was stopped (group B): 4 patients had a combination of acetylsalicylic acid (500 mg intravenous loading dose, then 100 mg oral dose once daily) and clopidogrel (300 mg loading dose followed by 75 mg dose once daily), one patient was given acetylsalicylic acid, and another received only clopidogrel. In group A, the inhibitory effect of tirofiban on cerebral microembolism was reversible in all but one patient, with an increase to a median MES rate of 13.5 (range, 0 to 35; P=0.001) per hour on TCD-II. In contrast, all patients of group B remained MES-negative at TCD-II recording (P<0.0001; Figure 2). In group A, the MES rate at TCD-II was significantly reduced when compared with iTCD (Figure 2).

In one patient acceding to repetitive on-off infusion, the inhibitory effect on cerebral microembolism was reversible in a reproducible manner during episodes of suspended tirofiban infusion. At reduced dosages, tirofiban incompletely suppressed the MES (Figure 3).

Discussion

Nature of MES: Solid or Gaseous?

In this study, we have shown for the first time that the nonpeptide platelet GPIIb/IIIa receptor antagonist tirofiban switched off cerebral microembolization in vivo at dosages determined from ex vivo platelet studies to be optimal for clinical use.14 Irrespective of the particular platelet-activating stimulus, the resulting platelet aggregation is mediated by GPIIb/IIIa receptors.13 These fibrinogen receptors are exclusively expressed on platelets. Activated GPIIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets thereby accomplishing the final step of platelet aggregation. Binding of GPIIb/IIIa receptor antagonists to platelets results in a highly selective and efficient inhibition of platelet aggregation independent from the respective platelet activating mechanism ("platelet anesthe- sia").14 At full dosage, tirofiban reversibly blocked MES, that is, the microembolization was "switched on and off," whereas at reduced dosages, tirofiban led to an incomplete suppression

Figure 1. Design of the study. There was no statistically significant difference between patient groups A and B concerning the length (median, range) of phase I (group A: 13.5, 2.5 to 22 hours; group B: 8.7, 2.3 to 22 hours) and phase II (A: 4, 2.5 to 67 hours; B: 10.5, 4 to 37 hours). iTCD, TCD-I, and TCD-II indicate initial, first, and second follow-up transcranial Doppler sonography, respectively. Pretreatment at iTCD varied among study patients, as outlined in text.

Results

All 24 patients (median age, 63; range, 32 to 80 years; 16 men) had recently been symptomatic, either with transient ischemic attack (n=4), amaurosis fugax (n=3), or ischemic stroke (n=18). The median time between qualifying neurological event and iTCD was 3 days (range, 0.1 to 37.5 days). Diagnostic workup excluded potential cardiogenic embolic sources and revealed arterial embolic origin in all patients
of MES (Figure 3). This is in accordance with the pharmacodynamic profile of a competitive, short-acting receptor antagonist. The fact that after cessation of tirofiban infusion the MES rate in some patients did not fully return to the initial rate is in line with previous observations suggesting that the MES rate in patients with carotid artery disease is not constant but declines over time. Since tirofiban is not expected to reduce embolization of atherosclerotic lipid and other matrix constituents, these factors virtually do not contribute to MES in our patients. Therefore, our results provide strong evidence that microemboli from atherothrombotic origin are solid, with platelet-fibrinogen units as predominant constituent parts. These findings are compatible with previous reports describing the effect of other antiplatelet agents on cerebral microembolization.

Platelet Activation Mechanism

In accordance with our observations, a substantial number of patients with carotid artery disease or early after CEA show MES or have ischemic symptoms irrespective of current antiplatelet therapy. Thus, we have to assume that in these cases, the medication is either not sufficient or not effective. In principle, common antiplatelet agents such as aspirin or thienopyridines do not fully inhibit aggregation of platelets activated through pathways different from thromboxane or ADP. Especially at sites of plaque inflammation, induction of tissue factor has been shown to play an important role in the destabilization of carotid artery stenosis. By its ability to bind factor VIIa, tissue factor directly activates the coagulation cascade with the generation of thrombin—a strong platelet activator—thereby linking plaque inflammation with arterial thromboembolism. The clinical relevance of these findings is illustrated by the observations of Goertler et al that aspirin application resulted only in partially reduced MES rates. Since one dose of aspirin blocks, almost completely and for several days the platelet's ability to aggregate through the thromboxane pathway, reversibility of the MES-blocking effect could not be examined. Here, irrespective of the pretreatment, the selective platelet GPIIb/IIIa inhibitor tirofiban resulted in a complete suppression of MES in all these cases. S-nitrosglutathione, an NO donor with relative platelet specificity, has also been shown to attenuate silent

<table>
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<th>Case</th>
<th>Sex, Age</th>
<th>Clinical Presentation</th>
<th>Embolic Source</th>
<th>Treatment at Time of iTCD</th>
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<th>TCD-I MES/h</th>
<th>TCD-II MES/h</th>
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TIA, Transient ischemic attack; A.f., amaurosis fugax; CCA, common carotid artery; ICA, internal carotid artery; UFH, unfractionated heparin; ASA, acetylsalicylic acid; MES, microembolic signals; iTCD, initial transcranial Doppler ultrasound; TCD-I, first follow-up TCD; TCD-II, second follow-up TCD; and CEA: carotid endarterectomy.

*CEA performed before TCD-II.
embolism after CEA and from symptomatic carotid plaques. In contrast to S-nitrosoglutathione, which may also interfere with monocytes and endothelial function, the selective platelet fibrinogen receptor antagonist tirofiban inhibits platelet aggregation in a highly efficient manner irrespective of the particular platelet activating mechanism. In acute coronary syndromes, GPIIb/IIIa receptor antagonists improve clinical outcome probably as the result of a substantially reduced microvascular obstruction with consequently diminished minor myocardial injury. In several animal models of focal brain ischemia, use of GPIIb/IIIa antagonists led to a substantial reduction in infarct size resulting from decreased microthrombosis. Hypoperfusion and embolism often coexist, and their pathophysiological features are interactive: Microvascular obstruction caused by arterial embolization leads to hypoperfusion with impaired clearance of emboli (for review, see Reference 27). As a result, ongoing microthrombosis caused by a sustained accumulation of fibrinogen and platelets leads to extension of ischemic brain damage. Recently, we have provided evidence that in patients with acute stroke, tirofiban prevents the transition of ischemic tissue into the infarct proper, as defined by MRI. The persistence of embolic signals is an independent predictor of recurrent transient ischemic attack or stroke. Thus, GPIIb/IIIa antagonists such as tirofiban may have a potential to bridge the ischemic risk in patients with unstable carotid disease. When compared with common antiplatelet agents, the highly efficient, short-acting GPIIb/IIIa inhibitors have the advantage of better steering properties, with median bleeding times returning to near-normal levels within 3 hours. This is especially important in critically ill patients when immediate surgical treatment, for example, craniotomy or CEA, is needed. At present, heparin is still widely used in patients with symptomatic carotid stenosis. Interestingly, at iTCD there were slightly higher MES rates in patients receiving heparin than those receiving antiplatelet agents (Table). At TCD-II, this difference became significant (Figure 2). This observation provides some further evidence that in symptomatic carotid artery disease, antiplatelet approaches may be superior to those using anticoagulation. In particular, a GPIIb/IIIa blockade might be used to passivate the embolic source followed by sustained suppression with oral antiplatelet agents. Currently available data concerning safety issues suggest that GPIIb/IIIa inhibitors, even when administered within a broad-window time frame, are not associated with a high cerebral bleeding rate in the setting of acute ischemic stroke. None of our patients who received tirofiban after CEA had bleeding complications. Recently, Koster et al provided evidence that the application of tirofiban appears to be safe even during cardiopulmonary bypass surgery.

**Limitations of the Study Design**

The unique pharmacodynamic profile of tirofiban enabled us to examine for the first time that in the individual patient, MES are reversibly blocked by a specific platelet fibrinogen receptor antagonist (Table, Figure 3). In our view, this partly compensates for the non-placebo-controlled study design. Nevertheless, because of the missing placebo group, we cannot estimate the impact of tirofiban in terms of relative or absolute risk reduction for asymptomatic microembolization at a given time point in our population.

**Conclusions**

Altogether, our observations strongly suggest that cerebral microembolism of arterial origin has the property of solid emboli, with platelet-fibrinogen units as predominant constituent parts. The short-acting GPIIb/IIIa antagonists may have the potential to bridge the ischemic risk in patients with unstable carotid disease.

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References

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