Effect of Therapy on Platelet Activating Factor–Induced Aggregation in Acute Stroke

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Platelet activating factor, a potent inducer of in vivo platelet activation and thrombosis, has been shown to be excessively active in acute ischemic stroke patients. Therefore, we studied the effect of aspirin/dipyridamole therapy in inhibiting platelet activating factor–induced platelet activation in acute ischemic stroke patients, 23 taking aspirin/dipyridamole and 21 untreated. Aspirin/dipyridamole-treated patients failed to show suppression of platelet activating factor–induced platelet aggregation even though collagen-induced activation was inhibited, suggesting that platelet activating factor acts by cyclooxygenase-independent mechanisms. Failure to suppress cyclooxygenase-independent mechanisms of platelet activation may explain the limited usefulness of current antiplatelet therapy, aspirin in particular, in stroke prevention. The role of selective platelet activating factor antagonists both in isolation and combined with aspirin needs to be investigated for their usefulness in the treatment and prevention of ischemic stroke. (Stroke 1989;20:609–611)

Exposure of vascular wall collagen is generally considered to be the initiating event for platelet activation in cerebral thrombosis. Therefore, a cornerstone of current antiplatelet therapy in stroke is the inhibition of collagen-induced platelet activation. We recently reported the results of platelet aggregation and secretion in a group of acute ischemic stroke patients treated with various antithrombotic agents (heparin, warfarin, and aspirin/dipyridamole). Only aspirin/dipyridamole treatment inhibited both collagen-induced platelet responses. Aspirin, however, has only limited usefulness in the prevention of cerebral ischemia. One reason for this limitation may be that cyclooxygenase inhibition does not suppress platelet responsiveness to all in vivo thrombogenic stimuli. Platelet activating factor (1-O-alkyl-2-O-acetyl-sn-glyceryl-3-phosphocholine) (PAF), a naturally occurring phospholipid derived mainly from stimulated leukocytes, is a potent inducer of platelet activation and thrombosis. Accordingly, we have extended our studies to explore the effect of aspirin/dipyridamole therapy on PAF-induced platelet aggregation.

Subjects and Methods

We studied 44 acute ischemic stroke patients with occlusive cerebrovascular disease admitted to Henry Ford Hospital. No patient had a known primary platelet or coagulation disorder. Ischemic stroke was diagnosed based on the sudden onset of a persistent focal neurologic deficit lasting >24 hours with no subsequent worsening and supported by computed tomography or magnetic resonance imaging. All patients with cerebral hemorrhage were excluded. All patients had small-volume subcortical infarcts believed to be secondary to small-vessel disease. Before study, patients were given either aspirin (333 ± 36 mg, mean ± SD) and dipyridamole (181 ± 46 mg) or no medication. Twenty-three (13 men, 10 women, mean ± SD age 65 ± 17 years) were given aspirin/dipyridamole and had 5.8 ± 4.0 days (mean ± SD) after the onset of the ictus; they had received medication for a minimum of 1 and a maximum of 10 days before our study. Twenty-one patients (12 men, nine women, mean ± SD age 65 ± 14 years) were unmedicated, and 3.4 ± 1.9 days (mean ± SD) had elapsed from the onset of neurologic dysfunction. All patients had small-volume subcortical infarcts believed to be secondary to small-vessel disease. Before study, patients were given either aspirin (333 ± 36 mg, mean ± SD) and dipyridamole (181 ± 46 mg) or no medication. Twenty-three (13 men, 10 women, mean ± SD age 65 ± 17 years) were given aspirin/dipyridamole and were 5.8 ± 4.0 days (mean ± SD) after the onset of the ictus; they had received medication for a minimum of 1 and a maximum of 10 days before our study. Twenty-one patients (12 men, nine women, mean ± SD age 65 ± 14 years) were unmedicated, and 3.4 ± 1.9 days (mean ± SD) had elapsed from the onset of neurologic dysfunction. There were 16 patients who suffered hypertension and seven smokers in each group, seven diabetics in the medicated and four in the unmedicated group. Antihypertensive and antidiabetic therapies were reasonably matched between groups.

Platelet aggregation was measured by the optical technique. Twenty milliliters of blood was collected into citric acid-citrate-dextrose (3.8% citrate) in a 1:10 dilution after the patient fasted or ate only a light, nonfat breakfast. Platelet-rich plasma (PRP)
was extracted by centrifugation at 180g for 15 minutes. For each experiment, 450 μl of PRP was warmed at 37°C for 2–3 minutes and stirred at 1,200 rpm using a Teflon-coated magnetic stirrer bar. The platelet count reached 250–400,000/mm³. No in vitro adjustments by dilution were made to achieve a fixed platelet count. PAF-acether (1-O-alkyl-2-O-acetyl-sn-glyceryl-3-phosphocholine), purchased from Avanti Polar Lipids, Alabama, was then applied in a volume of 3 μl. PAF was kept at −70°C in aliquots of 10 mg/ml chloroform. For each experiment an aliquot was blown dry under air and resuspended in Tyrode’s albumin buffer. It was our intention to study maximal responses to PAF; therefore, a final concentration of 1 mM was chosen arbitrarily. Aggregation was measured as a percentage of maximal light transmission. In all experiments, maximal light transmission was standardized using saline, which in our experience gave more consistent results than did platelet-poor plasma. The peak height of aggregation as percentage of the maximum recorded with saline was achieved rapidly (within 3 minutes) in all cases. The following measurements were made on each aggregation curve based on a modification of our previous studies: 1) aggregation height at 30 seconds after the addition of the inducing agent; 2) ED₅₀, time in seconds from the addition of the inducing agent to half-maximal aggregation (twice ED₅₀ was taken as the time to achieve peak aggregation); and 3) peak height, maximum extent of the aggregation wave.

Collagen-induced platelet aggregation of the same blood sample confirmed the inhibitory effect of aspirin/dipyridamole.

Student’s two-sample t test was used to compare aggregation responses between groups.

Results

Mean±SD PAF-induced aggregation responses in unmedicated and medicated stroke patients were aggregation height at 30 seconds (21±8% vs. 22±8% (p>0.38), ED₅₀ 38±24 seconds vs. 34±18 seconds (p>0.57), and peak height 43±25% vs. 39±19% (p>0.55). Thus, there was no evidence that aspirin/dipyridamole therapy inhibited PAF-induced platelet aggregation in this group of stroke patients.

Discussion

The triggering factor(s) initiating platelet activation in acute occlusive stroke is (are) unknown. Neutrophils, erythrocytes, and platelets are known to interact within thrombi: 7 of the putative mediators for this interaction, PAF may be pivotal. 8,9 PAF, derived mainly from stimulated leukocytes, is a potent inducer of platelet activation and thrombosis. 1,2,9 Furthermore, unlike collagen, which induces platelet activation only after several seconds, PAF induces platelet activation almost instantaneously. Hence, PAF may play a role in the pathogenesis of cerebral thrombosis, and therefore, suppression of its effect on platelets may be of therapeutic value.

In a recent study, only aspirin/dipyridamole among several antithrombotic treatments adequately inhibited collagen-induced platelet activation, most likely as a result of the aspirin effect. 1 Though aspirin suppresses collagen-induced platelet responses, it has only limited usefulness in the secondary prevention of ischemic stroke, raising the possibility that other thrombogenic stimuli may activate cyclooxygenase-inhibited platelets.

PAF-induced platelet aggregation in acute stroke patients was not suppressed by aspirin/dipyridamole therapy. Moreover, we recently observed that the PAF-induced increase in cytosolic ionized calcium concentration was also not suppressed by aspirin in acute stroke patients. 10 These observations suggest that PAF activates platelets independent of the cyclooxygenase pathway. 11 Oral dipyridamole in the doses we used does not consistently inhibit platelet phosphodiesterase activity and raise cyclic adenosine monophosphate levels 12 or inhibit adenosine diphosphate, 13 epinephrine, or collagen-induced platelet aggregation. 14 Moreover, there is no synergistic effect on platelet inhibition from the combination of aspirin and dipyridamole. 15 Therefore, the influence of dipyridamole on the results of our study is probably not significant.

PAF is a potent in vivo inducer of platelet activation, thrombosis, and ischemia. 16-23 Similar to that after collagen and thrombin, the increase in platelet cytoplasmic ionized calcium levels after activation with PAF in acute ischemic stroke patients was greater than normal. 10 Therefore, suppression of PAF-induced platelet activation, in addition to that provided by collagen and thrombin, may be necessary to more effectively limit thrombosis. Besides the platelet-mediated effects, PAF has been shown to cause direct neuronal damage, 14 cerebral vasoconstriction, 25 and cerebral hypoperfusion, 26 possibly mediated by specific PAF binding sites in the brain. 27 Derivatives from the Chinese herbal plant Ginkgo biloba have been shown to have selective PAF-antagonistic properties on human platelets both in vitro 28 and in vivo. 29 Furthermore, selective blockade of PAF receptors has been shown to improve recovery from brain ischemia. 30 Accordingly, selective PAF antagonists 31-33 such as derivatives of the Chinese herbal plants Caulis piperis futkadsurae (kadurenone) and G. biloba (BN50201, BN52063) and certain triazolobenzodiazepines and calcium channel blockers need to be investigated for their potential use in inhibiting PAF-induced responses in cerebral ischemia.

References


**Key Words** • aspirin • cerebrovascular disorders • platelet activating factor
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