Platelet Aggregation in Patients With Atrial Fibrillation Taking Aspirin or Warfarin

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**Background and Purpose:** Although warfarin and perhaps aspirin may be effective in preventing thromboembolism in patients with nonvalvular atrial fibrillation, some patients develop cerebral infarction despite these therapies. The purpose of this study was to determine inhibition of platelet aggregation in patients on aspirin and platelet reactivity in those on warfarin in the Stroke Prevention in Atrial Fibrillation study.

**Methods:** Twenty-four patients in the Stroke Prevention in Atrial Fibrillation study at the University of Illinois at Chicago, 17 on enteric-coated aspirin 325 mg/d and 7 on warfarin to produce an international normalized ratio of 2.0 to 4.5, had platelet aggregation studies performed during a 10-month period and interpreted by an investigator blinded to therapy. Epinephrine, adenosine diphosphate, collagen, and arachidonic acid were used as aggregating agents. Compliance was determined by pill count for those patients on aspirin.

**Results:** Seven patients taking aspirin had partial and 10 had complete inhibition of platelet aggregation. Three of seven patients on warfarin had hyperaggregable platelets. Compliance was 80% or greater for those patients taking aspirin. One patient on warfarin had partial inhibition of platelet aggregation.

**Conclusions:** Some patients in the Stroke Prevention in Atrial Fibrillation trial on aspirin 325 mg/d did not achieve complete inhibition of platelet aggregation. Others had hyperaggregable platelets. These findings suggest platelet-dependent mechanisms for aspirin and warfarin failure to prevent stroke in these patients. (Stroke. 1993;24:1458-1461.)

**KEY WORDS** • aspirin • atrial fibrillation • platelet aggregation • stroke prevention • warfarin

Warfarin has been shown to produce a relative risk reduction for brain and systemic embolism in patients with nonvalvular atrial fibrillation (NVAF) in five clinical studies. Through its inhibitory action on the production of functional clotting factors, warfarin is thought to prevent red clot formation in chambers where there is stasis, the presumed mechanism for thrombus formation in the heart in atrial fibrillation. Warfarin is not expected to inhibit platelet aggregation, however, and a thrombus itself is a stimulus for platelet adhesion and aggregation and additional thrombus growth. In addition, abnormal endocardial tissue surfaces and carotid stenosis may be found in patients with NVAF, suggesting platelet-dependent mechanisms for thrombus formation in this situation. Nonetheless, the value of warfarin anticoagulation for the prevention of stroke in patients with NVAF seems well established.

The efficacy of aspirin in the setting of NVAF, however, is less clear. Although aspirin therapy did significantly reduce stroke incidence in the Stroke Prevention in Atrial Fibrillation (SPAF) study, other studies have not shown a similarly beneficial effect; however, given the different doses of aspirin used in the studies, their results are difficult to compare. Recent studies suggest that different doses of aspirin may be needed to inhibit platelet aggregation and that a given dose of aspirin may not achieve the same effect in all patients.

Although the mechanisms that underlie the failure of aspirin or warfarin therapy to prevent stroke in NVAF patients are not fully understood, aspirin failure in some patients may be related to inadequate dose to achieve complete antiplatelet effect as well as to the lack of inhibition of the clotting mechanism, while failure in warfarin-treated patients may be related to lack of inhibition of platelet function, particularly when the patient has hyperaggregable platelets. To begin addressing these issues, the present study attempted to determine whether aspirin at the dosage given in the SPAF study (325 mg/d) could completely inhibit platelet aggregation and to determine the state of platelet reactivity in patients taking warfarin.

**Subjects and Methods**

Twenty-four patients in the SPAF study at the University of Illinois at Chicago had platelet aggregation studies performed during a 10-month period. All platelet aggregation studies were interpreted by the same...
Platelet aggregation in atrial fibrillation

FIG 1. Tracings of normal or hyperaggregable responses to platelet-aggregating agents. Responses shown are typical for 500 μmol/L arachidonic acid (AA) (free acid), 5 μmol/L adenosine diphosphate (ADP), 5 μmol/L epinephrine (EPI), and 0.8 μg/mL collagen (COL). Responses of hyperaggregable platelets look essentially the same but at lower concentrations of agonists.

 investigator (L.D.B.), who was blinded to medication. Seventeen patients were taking 325 mg/d of aspirin, and the other 7 patients were taking warfarin to achieve an International Normalized Ratio (INR) of 2.0 to 4.5. Institutional Review Board permission was obtained (No. 91-631).

Preparation of platelet-rich plasma (PRP) was performed in the following manner. Blood was drawn using 19- to 21-gauge butterfly needles, plastic syringes, and multiple syringe technique. Blood was immediately placed in plastic tubes containing citrate anticoagulant (9 parts blood to 1 part anticoagulant) and mixed. PRP and platelet-poor plasma (PPP) were prepared by standard centrifugation techniques at room temperature and kept sealed in plastic tubes until tested. A platelet count on the PRP was performed on an Abbott/Unipath CellDyne 3500SL (Abbott Park, Ill.), and if the count exceeded 400 000/μL, it was adjusted to approximately 350 000/μL with autologous PPP.

Platelet aggregation studies were performed using a model PAP4 aggregometer (BioData Corp, Hatboro, Pa) as previously described.9 Briefly, PRP was placed in each of four siliconized cuvettes and incubated for 1 minute at 37°C. An aggregating agent was then added to each tube, and aggregation was recorded for 10 minutes at 37°C with constant stirring at 1000 rpm. When saline was added to test for spontaneous aggregation, the recording time was extended to 20 minutes. If full (normal) aggregation responses were obtained with the initial concentrations of agents used (arachidonic acid [AA], 500 μmol/L; adenosine diphosphate [ADP], 5.0 μmol/L; epinephrine, 5.0 μmol/L; and collagen, 0.8 μg/mL), the aggregating agent was progressively titrated to the lowest concentration that produced a full response. If less than full responses were obtained with the initial concentrations, the concentration of each agent was doubled to determine whether full responses could be obtained. In interpreting aggregation responses and patterns, platelets were considered to show increased sensitivity to an agent when a full aggregation response was obtained with ≤100 μmol/L AA, ≤1 μmol/L ADP, ≤0.4 μg/mL collagen, or ≤0.5 μmol/L epinephrine. Platelets were considered to be hyperaggregable if increased sensitivity to more than one agent and/or the presence of spontaneous aggregation was detected (Fig 1). For patients taking aspirin, platelets showed partial inhibition when there was a full AA response, and/or the ADP response was normal, and/or epinephrine caused more than primary aggregation, and/or the collagen response was normal, slightly decreased, or decreased (Fig 2). Platelets were considered to show complete inhibition if there was no AA response, the ADP response was less than normal, epinephrine and collagen responses were absent or markedly decreased, and spontaneous aggregation was not detected (Fig 3).

Blood for prothrombin time/INR testing was collected by venipuncture into citrate anticoagulant, centrifuged to prepare PPP, and tested with a commercial thromboplastin reagent (International Sensitivity Index, 2.0) on an Ortho Koagulab 16S (Raritan, NJ) automated coagulation analyzer set to automatically calculate the INR. Results are reported as prothrombin time/control ratio as required by SPAF protocol.

For those patients on aspirin, compliance was determined by pill counts at the follow-up closest to the date of the platelet aggregation studies. For those patients on warfarin, the prothrombin time ratio (required by

FIG 2. Tracings show partial inhibition as a result of aspirin ingestion. ADP indicates 5 μmol/L adenosine diphosphate; EPI, 5 μmol/L epinephrine; COL, 0.8 μg/mL collagen; and AA, 500 μmol/L arachidonic acid. Note slow but steady rise in epinephrine-induced aggregation.

FIG 3. Tracings show complete inhibition as a result of aspirin ingestion. ADP indicates 5 μmol/L adenosine diphosphate; EPI, 5 μmol/L epinephrine; COL, 0.8 μg/mL collagen; and AA, 500 μmol/L arachidonic acid.
SPAF) represents that obtained at follow-up closest to the platelet aggregation study.

Results

In aspirin-treated patients, 7 of 17 patients had partial inhibition (Fig 2), and 10 patients had complete inhibition of platelet aggregation (Fig 3). Compliance ranged from 80% to 111% in these patients but was not always correlated with the degree of platelet aggregation inhibition. Three of 8 patients with less than 100% compliance had complete inhibition; 2 of 7 patients with greater than 100% compliance had partial inhibition of platelet aggregation.

Three of 7 patients taking warfarin had hyperaggregable platelets (Fig 1); 3 had normal platelet reactivity, and 1 had partial inhibition of platelet aggregation. Despite warnings to refrain from taking platelet-inhibiting agents while taking warfarin, we assume that the partial inhibition of platelet aggregation observed in 1 warfarin-treated patient resulted from inadvertent consumption of a compound containing aspirin or an aspirin-like agent.

Discussion

The Copenhagen AFASAK and the SPAF studies disagreed in their findings regarding the efficacy of aspirin compared with placebo for the prevention of stroke in patients with NVAF.1,2 The latter study showed a superiority of aspirin in this regard, with nearly a 50% risk reduction in favor of aspirin. A potential reason for the difference in aspirin efficacy is that a different dosage of aspirin was used in the Copenhagen AFASAK study (75 mg/d versus 325 mg/d in the SPAF study). Neither study dosed aspirin with regard to biologic efficacy, i.e., inhibition of platelet aggregation. Recent reports suggest that the ability of aspirin to affect platelet aggregation may depend on the dosage used as well as on the individual.9,10 The results of the present study suggest that some patients in the SPAF study who took 325 mg/d of aspirin did not achieve complete inhibition of platelet aggregation as measured by the methods described in the present study. Whether the failure to achieve inhibition of platelet aggregation in these individuals is clinically significant for prevention of stroke is unknown at this time. Likewise, it is unknown whether those AFASAK patients taking 75 mg/d failed to achieve inhibition of platelet aggregation.

The antithrombotic mechanisms of aspirin and warfarin are considered to be different. Whereas warfarin is considered to inhibit red clot (red blood cell fibrin-rich) formation in areas of stagnant flow in the atria or ventricle of the heart in a patient with NVAF, aspirin may be effective against white clot (platelet fibrin-rich) formation where there is concomitant atherosclerotic vascular disease of the carotid artery, mitral annulus calcification, or other endocardial damage.7,8 Platelet scintigraphy studies show active deposition of platelets on intracardiac thrombus.11 Although in one study warfarin was shown to inhibit this process, it is not expected to affect platelet aggregation per se, and the effect of aspirin on this process has not been shown.11 Hyperaggregable platelets were discovered in three of seven patients taking warfarin in the present study. While other studies have described alteration in hemostatic function and some platelet functions in patients with NVAF, we are unaware of any study that has shown platelet hyperaggregability to be present in these patients.12 Hyperaggregability of platelets may be indicative of a prothrombotic state that would not be expected to be relieved by or sensitive to warfarin therapy.13 In other studies, aspirin has been shown to decrease platelet hyperaggregability described in patients with other risk factors for stroke.14-16 Whether those patients with atrial fibrillation on warfarin but with hyperaggregable platelets would require concomitant antiplatelet therapy for prevention of thromboembolism is unknown at this time. Likewise, it is unknown whether any of the patients assigned to aspirin therapy had hyperaggregable platelets before aspirin ingestion or whether the presence of such platelet hyperaggregability relates to the incomplete inhibition of platelet aggregation demonstrated in seven of the 17 patients in this study.

In summary, at our center, aspirin at the dosage used in the SPAF study (325 mg/d) did not completely inhibit platelet aggregation in all patients. This finding is similar to that found in another study in which dose escalation was needed (sometimes up to 1300 mg) to achieve inhibition of platelet aggregation.9 In addition, increased platelet reactivity (platelet hyperaggregability) may be present in some patients with chronic NVAF, and this aspirin-sensitive mechanism for platelet-dependent thrombus formation would not be affected by warfarin. We hypothesize that the presence of hyperaggregable platelets in warfarin-treated patients and lack of complete inhibition of platelet aggregation in aspirin-treated patients may be related to medication failure in these patients.

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