Aspirin Response and Failure in Cerebral Infarction

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Background and Purpose: The purpose of this study was to assess the biological effect of aspirin as measured by the inhibition of platelet aggregation in patients taking aspirin for stroke prevention and in patients with acute stroke.

Methods: We administered increasing doses of aspirin (325, 650, 975, and 1,300 mg daily) to 113 patients for stroke prevention and measured the inhibition of platelet aggregation in these patients and in 33 patients with acute stroke taking aspirin before stroke onset.

Results: Eighty-five patients on ≤325 and six on ≥650 mg aspirin daily had complete inhibition of platelet aggregation. Increase of the dose by 325 mg in nine of the 22 patients with partial inhibition of platelet aggregation produced complete inhibition in five patients at 650 mg and in one at 975 mg. At 1,300 mg, three patients still had only partial inhibition of platelet aggregation (aspirin resistance). Of the 33 inpatients with acute stroke, 24 had platelet aggregation studies done before further administration of aspirin. Of these, 19 had complete inhibition of platelet aggregation and three had partial inhibition, with production of complete inhibition of platelet aggregation at dose escalation; one patient was aspirin-resistant and the other noncompliant.

Conclusions: How the inhibition of platelet aggregation relates to stroke prevention remains unclear. The ability of aspirin and the dose required to inhibit platelet aggregation may depend upon the individual. (Stroke 1993;24:345–350)

KEY WORDS • aspirin • cerebral infarction • stroke prevention

Recent studies have shown a reduction in risk of stroke from a daily intake of aspirin (ASA).1 However, the optimum dosage of ASA to achieve this end continues to be debated.2–4 Reasons for stroke occurrence despite ASA intake (ASA breakthrough or failure) remain obscure.4 Cerebral infarction might occur under these circumstances if the patient is noncompliant, has been on an inadequate dosage of ASA, is resistant to the ASA effect, or if the biological effect of ASA is irrelevant with regard to the specific pathogenic mechanism of cerebral ischemia. The purpose of the present study is to elucidate the predictability of ASA breakthrough and its cause.

Subjects and Methods

At the University of Illinois Hospital at Chicago two groups of patients were studied prospectively, those identified in the neurology outpatient clinic and those presenting with acute stroke. All patients had been taking ASA on a daily basis for secondary stroke prevention. Patients were excluded from data collection if there was documented noncompliance with medications or clinic appointments. Institutional review board approval (H-91-631) was obtained prior to patient identification.

Outpatients

For those patients identified in the outpatient clinic, the question to be answered was whether they had a measurable biological effect (inhibition of platelet aggregation) of ASA at a dosage already assigned by the primary care physician; if not, whether dosage increase could bring forth a full ASA effect.

Once identified, each patient was supplied with enteric-coated (EC) ASA by the investigators and kept on the same dosage he or she had been taking. Each patient was instructed to take that dosage of ASA daily, after breakfast, at 9 AM. On day 15, platelet aggregation studies were performed. Patients were to take their regular ASA dose at 7 AM the morning of the study. Patients were called the evening before and reminded of this. Tests of the prothrombin time and partial thromboplastin time and a complete blood count and a platelet count were also performed on the study day.

Depending on the result of the platelet aggregation study, the ASA dose was adjusted: if complete inhibition of platelet aggregation was documented, the pa-
Patient remained on the same ASA dose; if partial inhibition was documented, the patient's ASA dose was doubled and studies were repeated according to protocol until either the patient had documentation of the full antiaggregant effect or a dosage of 1,300 mg was achieved.

Patients were defined as having been on an inadequate ASA dose if upon initial testing partial inhibition of platelet aggregation was documented, but after increasing the ASA dose complete inhibition was found. Patients were defined as ASA-resistant if despite a daily dose of 1,300 mg ASA they never achieved complete inhibition of platelet aggregation. If the patient showed no inhibition of platelet aggregation at all, he was contacted and reminded to take his usual dosage and the test was repeated. If at repeat study partial or complete inhibition was present, then the patient was defined as probably noncompliant. Pills were counted at each visit to document compliance. For those patients found to be ASA-resistant, ticlopidine and warfarin were offered as alternative therapies where appropriate. Those patients found to have complete inhibition of platelet aggregation at a particular dosage of ASA were then continued on that dosage and observed for the occurrence of a cerebral ischemic event. No patient was continued on an ASA dosage known to partially inhibit platelet aggregation when alternative therapies were appropriate.

Inpatients

The second group of patients studied were those with acute stroke. For these patients the goal of the investigation was to determine the effect of ASA intake on platelet aggregation at the time of stroke. For these patients who were found by history to have been taking ASA on a daily basis for the prevention of cerebral ischemia before stroke onset, ASA was not to be given until platelet aggregation studies could be performed and interpreted. Where possible, this was done the next day. If no ASA effect or partial inhibition of platelet aggregation was found, the patient was defined as noncompliant, on the wrong ASA dosage, or ASA-resistant, depending on the results of platelet aggregation studies that were repeated until the patient was placed on a maximum of 1,300 mg daily of ASA supplied by the study investigators. If at resumption of the predmission ASA dosage the full antiplatelet effect was present, the patient was judged to have been noncompliant. If partial inhibition was present but on dosage increase complete inhibition appeared, then the patient was defined as having been on the wrong dosage of ASA. If despite an increase up to 1,300 mg ASA daily only partial or no inhibition of platelet aggregation was achieved, the patient was defined as ASA-resistant. If complete inhibition was present at initial testing after stroke occurrence and without the administration of ASA after stroke onset, the patient was defined as a true ASA failure. Patients found to be ASA failures or nonresponders (ASA-resistant) were offered alternative therapy (ticlopidine or anticoagulant) where appropriate.

Platelet Aggregation Studies

Blood was drawn from patients using a butterfly needle and multiple syringe technique, immediately placed into plastic tubes containing citrate anticoagu-

lant (nine parts blood to one part anticoagulant), and mixed. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by standard centrifugation techniques. A platelet count was performed on the PRP using a CellDyne 3000SL. If the platelet count of the PRP exceeded 400,000/μl, it was adjusted to approximately 350,000/μl using autologous PPP.

Platelet aggregation was measured according to the method of Born using a platelet aggregation profiler (model PAP4, BioData Corp., Hatboro, Pa) as previously described. Aliquots of PRP were incubated at 37°C for 1 minute before addition of the aggregating agent. The aggregating agents used were 500 μM arachidonic acid (AA), 5 μM adenosine diphosphate (ADP), 5 μM epinephrine, and 0.8 μg/ml collagen. Platelet aggregation was monitored for 8 minutes at 37°C with constant stirring at 1,000 rpm following addition of the aggregating agent. Depending on the responses obtained, agonist concentrations were adjusted upward or downward to determine platelet sensitivity.

AA (free acid) was obtained from Sigma Chemical Co., St. Louis, Mo., and was diluted in 75% ethanol:25% normal saline to a stock concentration of 50 mM; addition of 5 μl stock AA to 495 μl PRP resulted in a final concentration of 500 μM. ADP was obtained from Eastman Kodak Co., Rochester, N.Y., and was dissolved in sterile saline to a stock concentration of 500 μM; addition of 5 μl stock ADP to 495 μl PRP resulted in a final concentration of 5 μM. Epinephrine was obtained from Parke-Davis, Division of Warner-Lambert Co., Morris Plains, N.J., and was diluted in sterile saline to a stock concentration of 500 μM; addition of 5 μl stock epinephrine to 495 μl PRP resulted in a final concentration of 5 μM. Collagen was obtained from Organon Teknika Corp., Durham, N.C., and was dissolved in distilled water to a stock concentration of 16 μg/ml; addition of 25 μl stock collagen to 475 μl PRP resulted in a final concentration of 0.8 μg/ml.

All platelet aggregation studies were interpreted by the same investigator who was blinded to ASA dosage and other medication.

AA responses. Normal AA-induced platelet aggregation responses are dependent on intact cyclooxygenase and consequent metabolism of AA to thromboxane A2 (TXA2). In ASA-treated patients, AA responses are usually all-or-nothing depending on the degree of cyclooxygenase inactivation and hence the degree of inhibition of TXA2 formation. When AA failed to induce platelet aggregation at all concentrations tested, the result was reported as absent. (See Figure 1 for normal responses to all aggregating agents.)

ADP responses. ADP responses are only partially dependent on intact cyclooxygenase, and ADP gives dose-dependent responses whether or not the patient is treated with ASA. ADP-induced platelet aggregation was considered to be slightly decreased when 5 μM ADP caused maximal or near-maximal aggregation followed by a degree of disaggregation, moderately decreased when approximately 50% aggregation was followed by disaggregation, and markedly decreased when <50% aggregation was followed by disaggregation.

Epinephrine responses. Epinephrine is capable of producing a small degree of platelet aggregation (primary aggregation) independent of cyclooxygenase activity.
However, secondary aggregation is dependent on the release reaction induced by TXA2 and requires metabolism of AA (released from membrane phospholipids) by cyclooxygenase. Epinephrine-induced platelet aggregation was considered to be absent when 5 μM epinephrine caused ≤20% aggregation (primary aggregation only), markedly decreased when 20–30% aggregation was observed, decreased when 30–50% aggregation was observed, and slightly decreased when 50–65% aggregation was observed.

Collagen responses. Collagen-induced platelet aggregation is primarily dependent on intact cyclooxygenase. Collagen-induced aggregation was considered to be absent at <10% aggregation, markedly decreased at 10–20% aggregation, decreased at <50% aggregation, and slightly decreased at 50–65% aggregation.

Spontaneous aggregation. Spontaneous aggregation of platelets (an index of hyperaggregable platelets) caused by the addition of saline vehicle and stirring in the aggregometer was determined. Spontaneous aggregation was considered to be absent when ≤10% aggregation was observed 20 minutes after addition of vehicle.

**Aspirin Efficacy**

ASA treatment was considered to be fully effective (Figure 2) when AA response was absent; ADP response was slightly, moderately, or markedly decreased; epinephrine response was absent or markedly decreased; collagen response was absent or markedly decreased; and spontaneous aggregation was absent. ASA treatment was considered to be less than fully effective (Figure 3) when AA caused aggregation and/or ADP response was completely normal and/or epinephrine response was normal, slightly decreased, or decreased and/or collagen response was normal, slightly decreased, or decreased.

**Results**

From October 1991 through June 1992, 113 outpatients were prospectively identified. Each was receiving EC ASA for stroke prophylaxis at the time of identification. The population consisted of 46 men and 67 women aged 61.8±12.7 and 60.1±12.8 years, respectively; 72 patients were black, 25 were Caucasian, 15 were Hispanic, and one was Oriental. One hundred seven patients received ≤325 mg EC ASA daily; at this dosage inhibition of platelet aggregation was complete in 85 patients and partial in 22. Twelve patients were lost to follow-up, and one patient was converted to warfarin therapy. Escalating the dosage of EC ASA to 650 mg daily resulted in complete inhibition in five of the remaining nine patients. In the four without complete inhibition, further increase in EC ASA to 975 mg daily caused complete inhibition in only one patient. Of the remaining three patients, at 1,300 mg EC ASA daily, all still had only partial inhibition (ASA resistance). There were six patients who received ≥650 mg ASA at study onset. All six of these patients had complete inhibition of platelet aggregation at this ASA dosage.

Of the 33 patients who presented with acute stroke, 24 had platelet aggregation studies done before further administration of ASA. Of these 24, 19 had complete
and three had partial (with complete inhibition at dose escalation) inhibition of platelet aggregation at the time of the stroke. One patient was ASA-resistant and one was noncompliant (rechallenge with the prestroke ASA dosage produced complete inhibition). Nine patients had no platelet aggregation study done before the next ASA dose was given inadvertently. These patients were considered ASA failures by history only because platelet aggregation study results did not reflect the patient’s platelet aggregation status at the time of the stroke.

Of the 113 patients taking ASA for stroke prevention, eight developed cerebral infarction after identification. Of these eight, at the time of stroke five had complete and three had partial inhibition of platelet aggregation. Of the three with partial inhibition, two had partial and one had complete inhibition at testing before stroke onset.

**Discussion**

While debate has centered around the potential favorable efficacy of lower-dose and unfavorable side effects of higher-dose ASA for the prevention of stroke, it must be noted that doses of 1,300 as well as 30 mg daily have been shown to reduce the risk of cerebral ischemia. Preference for a lower ASA dose is due to potentially fewer gastrointestinal side effects and theoretical, but controversial, reversible and undesired cyclooxygenase inhibition at the vascular wall. Higher ASA doses are thought to cause a prothrombotic condition due to the latter mechanism. At least one study has suggested that ASA is ineffective at higher dosage. Other studies suggest that higher ASA doses may be needed to achieve more complete inhibition of platelet aggregation, a long-lasting effect, and other antithrombotic effects. Despite the efficacy of ASA for stroke prevention, the fact remains that some patients develop cerebral infarction despite ASA intake. The reasons for this are unclear. The results of the present study suggest that noncompliance is rare and that certain individuals develop a biological effect of ASA (inhibition of platelet aggregation) at a different but greater ASA dose and that others may never respond in this manner to ASA. The significance of achieving a measurable biological effect of ASA with regard to the prevention of cerebral ischemia, however, remains uncertain.

In this study, inhibition of platelet aggregation was used to define the biological effect of ASA. This effect occurs due to inhibition of platelet cyclooxygenase and TXA₂ production. Four aggregation stimuli (AA, ADP, epinephrine, and collagen) were used to determine a full profile of the ASA effect on platelet reactivity. Thrombin was not used in this study because in PRP the concentration of thrombin needed to stimulate platelet aggregation is near that which causes clot formation via the direct action of thrombin on fibrinogen.

While plasma thromboxane B₂ (TXB₂) concentrations can be measured, basal levels are generally very low and preclude detecting decreases in plasma TXB₂ as an index of the ASA effect in reducing platelet reactivity. We did not measure prostaglandin metabolites in our patients. However, in a recent study, Tohghi et al measured serum thromboxane generated during the clotting of unanticoagulated blood in vitro and showed that serum TXB₂ generation decreased significantly after 40 mg ASA per day and decreased further with increasing doses of ASA. The authors also showed that urinary 11-dehydro-TXB₂, decreased by 42% after 40 mg/day, by 78% after 320 mg/day, and by 91% after 1,280 mg/day. Urinary 2,3-dinor-6-ketoprostaglandin F₁α (the major metabolite of prostacyclin) was not significantly decreased at 40 mg/day but did decrease significantly with increasing doses of ASA. While it could be argued that this represents a detrimental effect of higher ASA dosage, it may well be that any decrease in prostacyclin synthesis is counterbalanced by the more effective inhibition of platelet reactivity.

While ASA ingestion is known to cause an increase in the bleeding time, the increase is not consistent from patient to patient. In addition, the bleeding time is technique-dependent and frequently not reproducible, and, in our experience, a prolonged bleeding time may cause keloid formation in blacks. Therefore, we did not study bleeding times in our patients. Likewise, we did not measure serum salicylate levels because they reflect absorption rather than biological effect and we are unaware of any study that has shown that ASA is not completely absorbed.

While some studies have measured decreases in TXB₂ generation and/or platelet aggregation in patients taking ASA for stroke prevention, no study has shown that the presence of these effects is important for stroke prevention when compared with their absence or other biological effects of ASA. In the Stroke Prevention in Atrial Fibrillation (SPAF) Study, the biological effect of warfarin was measured by the international normalized ratio; ASA’s mode of action was not measured. While ASA inhibits cyclooxygenase-dependent pathways of platelet activation, it does not inhibit cyclooxygenase-independent pathways (such as thrombin-induced platelet activation). In addition, ASA does not affect the binding of ligands other than fibrinogen to platelet glycoproteins IIb/IIIa (for example, von Willebrand factor). This latter mechanism of platelet activation may be induced by shear force alone. Shear force may be higher where there is arterial stenosis or arteriolar narrowing such as in tight internal carotid artery (ICA) bifurcation stenosis or intracranial small-vessel lipohyalinotic or atheromatous disease. The results of the North American Symptomatic Carotid Endarterectomy Trial (NASCET), in which nearly 90% of patients randomized to medical therapy were on ASA for stroke prevention, are consistent with the theory of ASA inefficacy for the prevention of shear-induced platelet aggregation. For symptomatic patients with ≥70% stenosis at the ICA bifurcation, carotid endarterectomy followed by ASA therapy was better than ASA alone. Under conditions of lesser degrees of stenosis and lower shear forces but injured endothelium, ASA would be expected to prevent platelet-initiated thrombosis. No study to date has addressed the efficacy of ASA for stroke prevention in patients with intracranial small-vessel disease. Mori et al suggested that platelets from insulin-dependent diabetics were resistant to the effect of ASA by a mechanism independent of both cyclooxygenase and platelet activating factor pathways of aggregation. Whether this is due to microangiopathy-related shear force is uncertain. However, using in vitro studies of inhibition of shear-induced platelet aggregation in blood from patients
taking ASA, Ratnatunga et al\(^{33}\) showed that >300 mg/day will affect this mechanism of thrombosis. In the present study, the biological effect of ASA on shear-induced platelet aggregation was not measured because it is not reflected by platelet aggregation studies using AA, ADP, collagen, or epinephrine. It is therefore impossible to state that those patients defined as ASA failures in the present study had all antiaggregant effects of ASA at work at the time of stroke onset. Finally, because ASA is an inhibitor of one pathway for the stimulation of platelet activation and recruitment, platelet activation and recruitment may continue in response to thrombin and platelet-released substances (ADP, serotonin) despite complete inhibition of the cyclooxygenase pathway by ASA.\(^{19}\) This fact may also explain the mechanism of ASA failure to prevent stroke.

Platelet aggregation may be increased under the circumstance of acute stroke.\(^{34-36}\) Despite this, the presence of complete inhibition of platelet aggregation in those patients defined as ASA failures suggests that the results of the present study were not affected by this phenomenon. Daily variation of platelet aggregation, with morning and afternoon peaks, has been reported in healthy individuals.\(^{37}\) Because platelet aggregation studies were performed at the same time of day (before noon) in all patients in this study, it is doubtful that diurnal variation affected conclusions regarding the presence or absence of an ASA effect when one patient is compared with another.

No study before the present one has indicated that there may be patients who are ASA-resistant. While the maximum dosage used to define ASA resistance was 1,300 mg/day, it is not known whether inhibition of platelet aggregation would have been achieved had a higher dosage of ASA been given. Likewise, it is unknown whether resistance in this study was due to problems at the platelet/ASA level of interaction, to faulty absorption, or to some other mechanism.

At present conclusions regarding the mechanism of efficacy of ASA for stroke prevention cannot be made from any of the large randomized trials already reported. No study has used doses of ASA to achieve platelet antiaggregant or another effect and compared efficacy under this condition with efficacy under its absence. While it is clear that the dosing of ASA without regard to its biological effect can reduce some risk of stroke, the preventive effect of ASA may be underestimated. In addition, strict identification of subgroups of patients defined by risk factors for stroke, such as has been done in the SPAF study and NASCET, may better elucidate the efficacy of ASA with regard to pathogenic mechanism and the role of platelet aggregation in each instance. The present study is ongoing to determine the importance of inhibition of platelet aggregation for the prevention of stroke in patients with known risk factors for cerebral ischemia.

**Acknowledgments**

The authors gratefully acknowledge the excellent technical expertise of JoAnne Walker and Raquel Martinez in performing platelet aggregation testing on the study patients. We would also like to thank Jeanne Ward for her skills in preparing this manuscript.

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Aspirin response and failure in cerebral infarction.
C M Helgason, K L Tortorice, S R Winkler, D W Penney, J J Schuler, T J McClelland and L D Brace

Stroke. 1993;24:345-350
doi: 10.1161/01.STR.24.3.345

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