Development of Aspirin Resistance in Persons With Previous Ischemic Stroke

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Background and Purpose The ex vivo effect of aspirin (ASA) on platelet aggregation, the platelet component of thrombosis, was studied at repeated intervals in a cohort of patients taking aspirin for recurrent ischemic stroke prevention to define the maintenance of efficacy over time.

Methods We administered increasing doses of aspirin (from 325 to 1300 mg/d) to patients with previous ischemic stroke and determined the extent of inhibition of platelet aggregation after 2 weeks and thereafter at approximately 6-month intervals.

Results Over 33 months, 306 patients had platelet aggregation studies performed to define their initial response to ASA therapy. Of these, 228 had complete and 78 had partial inhibition of platelet aggregation at initial testing. To date, 119 of those who had complete inhibition and 52 who had partial inhibition have undergone repeat testing at least once. At repeat testing 39 of the 119 (32.7%) with complete inhibition at initial testing had lost part of the antiplatelet effect of ASA and converted from complete to partial inhibition without change in ASA dosage. Of the 52 with partial inhibition at initial testing, 35 achieved complete inhibition either by ASA dosage escalation (in 325 mg/d increments) or fluctuation of response at the same dosage, but 8 of those 35 (22.8%) had reverted to partial inhibition when tested again. Overall, 8.2% of patients ultimately exhibited ASA resistance to 1300 mg/d—8 of 52 (15.4%) with partial inhibition and 6 of 119 (5.0%) with complete inhibition at initial testing.

Conclusions The antiplatelet (and presumably the antithrombotic) effect of a fixed dose of ASA is not constant over time in all individuals. The mechanisms by which increased dosage requirement or ASA resistance develops and the clinical significance of this development are currently undefined. (Stroke. 1994;25:2331-2336.)

Key Words • aspirin • aspirin resistance • platelet aggregation • stroke prevention

At present, a fixed dose of aspirin (ASA), usually 80 or 325 mg/d, is used for the prevention of recurrent ischemic stroke. However, unlike the case with other antithrombotic agents, it is not customary to measure the level or any biological effect of ASA to ensure adequate individual dosing or to use such a measurement to ensure that the efficacy of the dosage is maintained over time. Although the argument for prescription of low- versus high-dosage ASA relies on theoretical considerations regarding the prostacyclin/thromboxane A2 ratio, no large randomized study has correlated any effect of ASA dosage on this ratio with clinical outcome. Grotemeyer and colleagues1 modified the platelet reactivity test of Wu and Hoak to identify patients with higher than usual platelet reactivity 12 hours after ingestion of 500 mg ASA, and called such patients "secondary aspirin nonresponders." They predict that the finding of secondary nonresponse to ASA identifies patients at high risk for recurrence of vascular events.

In a pilot study of patients taking ASA for prevention of recurrent stroke we found that different subjects required different ASA dosages to achieve complete inhibition of platelet aggregation.2 Similar findings have been reported by Hormes and colleagues,3 who showed that in a cohort of selected normal individuals the dosage required to inhibit aggregation of platelets or cause disaggregation ex vivo varied for each subject.

One meta-analysis of recurrent ischemic stroke risk reduction and ASA dosage indicates that higher dosages of ASA may be therapeutically more effective.4 Other studies with low-dosage ASA suggest that it may be as clinically effective as slightly higher dosages.5,6 Despite these results, no study offers any explanation for or prediction of failure of ASA treatment in a given person; very low dosages have not been compared with much higher dosages, and no measure other than compliance has been used to ensure continued efficacy of ASA in a given patient over a study's duration.

Unlike any previous work done in this area, the present study involves following over time a biological marker for the antiplatelet efficacy of ASA in a large cohort of patients with previous ischemic stroke. We have found that a fixed dose of ASA is not sufficient to completely inhibit platelet aggregation in all individuals. In addition, we have observed that some patients are resistant to ASA in this regard and that a given person’s ASA dosage requirement may vary over time.2,7 Some individuals develop a progressively increasing dosage requirement, ASA resistance, or paradoxical platelet hyperactivity in response to the doses of ASA currently in clinical use.
Subjects and Methods

University of Illinois at Chicago Institutional Review Board (IRB) approval was obtained, and patient consent and study procedures were carried out in accordance with the IRB guidelines.

From October 1991 through June 1994, a period of 33 months, 306 patients taking ASA for prevention of recurrent stroke underwent initial platelet aggregation studies. Two weeks or more after the subjects began taking 325 mg/d or, in a few cases, higher doses, platelet aggregation studies were performed as previously described to define the degree of platelet inhibition. If platelet aggregation was less than complete, ASA dosage was escalated by 325 mg/d and aggregation studies performed again after approximately 2 weeks. This procedure was repeated until complete inhibition was achieved or until a dosage of 1300 mg/d was reached without complete inhibition. When complete inhibition was achieved, the patient was continued on that dosage and scheduled for repeat testing approximately 6 months later. For patients in whom only partial inhibition was achieved at 1300 mg/d, ASA therapy was continued, but the dosage was adjusted downward to 325 mg/d or to the lowest ASA dosage that gave the same degree of inhibition of platelet aggregation as 1300 mg/d. In patients who developed resistance to a given dosage of ASA (this resistance being defined as partial inhibition of platelet aggregation at a dosage that previously caused complete inhibition), dosage escalation was carried out in the same manner as described above. However, before dosage escalation, we attempted to repeat the platelet aggregation studies after 2 more weeks on the same ASA dosage and after reminding the patient of the importance of compliance.

Only patients with previous ischemic stroke who had been prescribed ASA for recurrent stroke prevention by either his or her primary care physician or neurologist were eligible for this study. Each patient was supplied with the same brand of enteric-coated ASA by the investigators (Time Cap Labs, Inc) and continued on the same dosage he or she had been taking as already assigned by the primary care physician. Each patient was instructed to take that dosage of ASA daily, after breakfast, at 9:00 AM. After at least 2 weeks, platelet aggregation studies were performed. Patients took their regular ASA dose at 7:00 AM the morning of the study, having been called the evening before and reminded to do this. After blood was drawn, each patient reported to the study nurse for compliance procedures. Interpretation of aggregation results was made according to our previously published criteria. Of the 306 patients in the present study, 228 had complete inhibition of platelet aggregation and 78 had partial inhibition at initial testing. Thirty-nine of 119 (32.7%) patients who had complete inhibition of platelet aggregation at initial testing had, after reinforcement of dosing instructions, complete inhibition at the same dosage at repeated testing or when, by pill count at the 3-month follow-up, there was not 100% correlation of the expected number of pills remaining with the actual number remaining.

Results

Of the 306 patients in the present study, 228 had complete inhibition of platelet aggregation and 78 had partial inhibition of platelet aggregation at initial testing. To date, 171 patients have undergone repeat testing at 6-month intervals at least once; 119 of these had complete inhibition and 52 had partial inhibition of platelet aggregation at initial testing. Thirty-nine of 119 (32.7%) patients who had complete inhibition of platelet aggregation at the initial prescribed dosage of ASA...
lost this effect over time and at repeated testing had only partially inhibited platelet aggregation (Tables 1 and 2). Thirty-five of the 52 patients whose platelet aggregation was partially inhibited at initial testing eventually achieved complete inhibition of platelet aggregation by either dosage escalation or fluctuation of response at the same dosage. Eight of those 35 (22.8%) patients reverted to partial inhibition over time when tested again (Table 3). In summary, of the 154 subjects who were at one time on a dosage of ASA sufficient to completely inhibit platelet aggregation, 47 (30.5%) did not maintain that effect at repeated testing despite fulfilling the criteria of regular compliance checks.

Several patterns of decreased efficacy of ASA as measured over time by platelet aggregation studies have emerged. Of the patients who lost efficacy having once obtained complete inhibition of platelet aggregation, 21 of 47 (44.6%) had fluctuations of this effect. Others developed hyperaggregability or spontaneous aggregation in the course of fluctuation or as part of an increased dosage requirement. Eight patients had complete inhibition of platelet aggregation, 47 (30.5%) did not maintain that effect at repeated testing despite their dosage escalation to a daily dose of 1300 mg. Eight of 52 (15.4%) whose inhibition was initially only partial and 6 of 119 (5%) whose inhibition was initially complete and who underwent repeated testing (a total of 14 of 171 patients, or 8.2%) were deemed ASA resistant (Tables 1 through 4).

**Discussion**

The antithrombotic efficacy of ASA is predicated on it's irreversible inhibition of cyclooxygenase in the platelet; this inhibition is in turn responsible for decreased thromboxane A2 production and inhibition of platelet aggregation. A possible unfavorable effect of ASA is its inhibition of prostacyclin (a vasodilator and platelet antiaggregant) production by the endothelium. In a recent study by Tohgi et al,9 a dose-dependent response to ASA was reflected by urinary 11-dehydrothromboxane B2 excretion. These authors showed that urinary 11-dehydrothromboxane B2 levels decreased by 42% after 40 mg/d ASA, by 78% after 320 mg/d ASA, and by 91% after 1280 mg/d ASA. Prostacyclin production was not significantly decreased with 40 mg/d ASA as measured by its major metabolite, urinary 2,3-dinor-6-ketoprostaglandin F1α. Prostacyclin production did decrease significantly with increased doses of ASA. Despite these results, it must once more be emphasized that the clinical effect of ASA has never been correlated with a measurable biological effect in any of the large randomized trials of the efficacy of ASA in prevention of recurrent stroke. On the other hand, the study by Tohgi et al also showed that when another test of ASA efficacy, inhibition of platelet aggregation, was applied, increasing dosages of ASA were needed to achieve this effect when increasing dosages of ADP were used as the agonist.

The effect of ASA on prostacyclin production may not always be linearly related to dose. In a recent study, Gow and colleagues10 showed that when the ratio of prostacyclin to thromboxane A2 metabolite was measured in a normal adult population, a daily dose of 80 mg or 325 mg ASA was more favorable than alternate-day doses of ASA. On the other hand, the study by Tohgi et al also suggested that there was a more linear relation between ASA dosage and its effect on prostacyclin and TXA2 metabolites. Their study did not address whether the inhibition of platelet reactivity by ASA could counterbalance or override any significance of the suppression of prostacyclin production in terms of antithrombotic effect. It is noteworthy that the endothelium produces vasoconstrictor prostanoids and that this production may be inhibited by ASA.11,12 Theoretically this latter effect of ASA may be desirable, but its potential benefit in the setting of cerebral ischemia is unknown. The endothelium also produces important platelet inhibitors in addition to prostacyclin. Nitric oxide (NO) is one of these and may be more potent than prostacyclin in this regard.13 Currently it is considered unlikely that ASA effects NO production. Thus, even if ASA inhibits prostacyclin production, the endothelial production of NO is unaltered.
TABLE 2. Increased Aspirin Dosage Requirement and Development of Aspirin Resistance

<table>
<thead>
<tr>
<th>Patient</th>
<th>Starting Aspirin Dosage (mg/d)</th>
<th>Platelet Aggregation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>325</td>
<td>C→P † →P(HA)(SA)650</td>
</tr>
<tr>
<td>23</td>
<td>80</td>
<td>C † →P(SA)(325)→P</td>
</tr>
<tr>
<td>24</td>
<td>325</td>
<td>C→(SA)† →P1200 R</td>
</tr>
<tr>
<td>25</td>
<td>325</td>
<td>C→N→P † →C(650)→N(HA) † →C(975) † →P(1300) R</td>
</tr>
<tr>
<td>26</td>
<td>325</td>
<td>C→P→P</td>
</tr>
<tr>
<td>27</td>
<td>325</td>
<td>C→P † →C(650)→P † →P(SA)(975) † →P(1300) R</td>
</tr>
<tr>
<td>28</td>
<td>650</td>
<td>C→P † →P(975) † →P(1300) ↓ →P(SA)325→P(SA)→P(SA) R</td>
</tr>
<tr>
<td>29</td>
<td>325</td>
<td>C→C→C→P † →C(650)→C→C</td>
</tr>
<tr>
<td>30</td>
<td>325</td>
<td>C→P † →C(650)</td>
</tr>
<tr>
<td>31</td>
<td>325</td>
<td>C→C→C→C→P→P</td>
</tr>
<tr>
<td>32</td>
<td>325</td>
<td>C→C→C→P † →C(650)→C→C</td>
</tr>
<tr>
<td>33</td>
<td>325</td>
<td>C→C→P † →C(650)→C</td>
</tr>
<tr>
<td>34</td>
<td>325</td>
<td>C→P † →P(650) † →P(SA)(975) † →P(SA)(1300)(stroke) R</td>
</tr>
<tr>
<td>35</td>
<td>650</td>
<td>C→P † →P(325) † →C(650)→C</td>
</tr>
<tr>
<td>36</td>
<td>325</td>
<td>C→P † →C(650)</td>
</tr>
<tr>
<td>37</td>
<td>325</td>
<td>C→P † →C(650)→C→C</td>
</tr>
<tr>
<td>38</td>
<td>325</td>
<td>C→P † →C(650)</td>
</tr>
<tr>
<td>39</td>
<td>325</td>
<td>C→P † →P(650) † →P(975) ↓ →P(1300) ↓ →P(325) R</td>
</tr>
</tbody>
</table>

C indicates complete inhibition of platelet aggregation; P, partial inhibition of platelet aggregation; HA, hyperaggregability; SA, spontaneous aggregation; R, resistance to a given aspirin dose (up to 1300 mg); N, normal platelet aggregation; †→, platelet aggregation without increase or decrease in aspirin dosage; † →, platelet aggregation with increase in aspirin dosage; and ↓ →, platelet aggregation with decrease in aspirin dosage. Numbers in parentheses indicate revised aspirin dosage.

Time interval between testing during dose escalation or dose reinforcement was typically 2 weeks. Other testing intervals were typically 6 months.

Reduction in aspirin effect due to decreased dosage.

rendering any effect of ASA on prostacyclin perhaps irrelevant with respect to platelet inhibition.

The present study focused on the platelet component of thrombosis and the anti-thrombotic effect of ASA on the platelet as measured by inhibition of platelet aggregation ex vivo to physiologically important activating agents (ADP, epinephrine, and others). The effect of ASA on prostacyclin and other measures of platelet sensitivity were not measured. A theoretical problem with this approach is that ASA and other nonsteroidal anti-inflammatory agents (ibuprofen or indomethacin, inhibit cyclooxygenase-mediated production of prostacyclin and thromboxane; with use of these agents the metabolism of cyclooxygenase's substrate, AA, is shifted to the lipoxygenase pathway. Products of that pathway, such as leukotrienes, may cause vasoconstriction. However, other products of the lipoxygenase-mediated conversion of AA to cyclooxygenase, 15-hydroperoxyeicosatetraenoic acid (15-HPETE) and 15-hydroxyeicosatetraenoic acid (15-HETE), may have antiplatelet effects.13

There is some evidence that newer antiplatelet agents may be as effective as ASA alone for recurrent stroke prevention. However, these agents (eg, ticlopidine) are costly and have side effects of their own, and it is not clear that the benefits of those agents exceed those offered by ASA. The present study did not describe the pharmacokinetics of enteric-coated ASA. In other studies describing ASA nonresponders as well as dose-dependent differences in the measured biological effect of ASA, enteric-coated ASA was not used.11,13,14 We therefore do not suspect that the similar findings in our study are related to ASA preparation. Indeed, no difference in platelet cyclooxygenase activity has been found when the latter is measured by in vitro radiometric technique when enteric-coated ASA is compared with compressed-ASA preparations.15 A recent study has shown inhibition of platelet aggregation and thromboxane synthesis after ingestion of enteric-coated ASA.16

Although other medications commonly used in patients with risk factors for stroke can affect platelet reactivity, it is expected that these effects only cause more inhibition of platelet aggregation than might be attributable to the effect of ASA dosage alone. Thus, complete inhibition of platelet aggregation may erroneously be judged to be due solely to a person's ASA intake. This may be one explanation for the observation of fluctuation of ASA's efficacy in some patients and may expose these patients to thrombotic risk during periods of partial inhibition of platelet aggregation.

This study has raised important questions about the way ASA is used for secondary prevention of stroke. First, in the present study there were subjects in whom there was a fluctuation of ASA's effect at the same dosage over time (Tables 1 and 3). Several of these
patients had recurrent stroke at a time when platelet aggregation was not completely inhibited. While one may argue that fluctuation of ASA’s effect, development of increasing dosage requirement, or resistance are due to noncompliance, the fact remains that the effect of ASA in these subjects was incomplete during the time they were taking ASA for prevention of ischemic stroke. Whatever the cause, this incomplete effect may be a reason for the failure of ASA to prevent stroke in some patients. Second, the mechanisms of increased dosage requirement, development of ASA resistance, or platelet hyperaggregability remain uncertain at this time. The possibility that ASA dosage needs to be adjusted to ensure continued efficacy, as is done with other medications such as warfarin, is clear. The potential increased risk of thrombosis due to increased platelet reactivity if ASA is suddenly discontinued remains a theoretical possibility. In patients who apparently no longer have the full antiplatelet aggregation effect of ASA, the potential need for the addition of or change to another antithrombotic agent remains real. It is presently unknown whether a “drug holiday” (a period during which ASA is not taken, followed by a resumption of dosage) could restore platelet response to ASA. Finally, the response to ASA at dosages higher than 325 mg/d has not been described. Most likely the answer lies in the mechanism of resistance.

The implications of this study may be applicable to the use of other and newer antiplatelet agents. Once antiplatelet therapy has been chosen for use to prevent recurrent ischemic stroke (whether in the clinical or study situation), it may be desirable to ensure a measurable desired biological effect at the onset and at recurrent ischemic stroke (whether in the clinical or study situation), it may be desirable to ensure a measurable desired biological effect at the onset and at repeated intervals during the course of therapy. We feel that the therapeutic benefit of ASA can be optimized by dosing according to biological effect rather than by fixed low or high dosage.

References


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