Effect of Low-Intensity Warfarin Anticoagulation on Level of Activity of the Hemostatic System in Patients With Atrial Fibrillation

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Background and Purpose: The Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF) demonstrated that low-intensity warfarin anticoagulation can, with safety, sharply reduce the rate of stroke in patients with nonvalvular atrial fibrillation. The beneficial effect of warfarin was presumably related to a decrease in clot formation in the cardiac atria and subsequent embolization.

Methods: To assess the effect of warfarin therapy on in vivo clotting in patients in the BAATAF, we measured the plasma level of prothrombin activation fragment F1+2. One sample was obtained from 125 patients from the BAATAF; 62 were taking warfarin and 63 were not taking warfarin (control group).

Results: The warfarin group had a 71% lower mean F1+2 level than the control group (mean F1+2 of 1.57 nmol/L in the control group compared with a mean of 0.46 nmol/L in the warfarin group; P<.001). F1+2 levels were higher in older subjects but were consistently lower in the warfarin group at all ages. Fifty-two percent of patients in the control group were taking chronic aspirin therapy at the time their F1+2 level was measured. Control patients taking aspirin had F1+2 levels very similar to control patients not taking aspirin (mean of 1.52 nmol/L for control patients on aspirin compared with 1.64 nmol/L for control patients off aspirin; P>.1).

Conclusions: We conclude that prothrombin activation was significantly suppressed in vivo by warfarin but not aspirin among patients in the BAATAF. These findings correlate with the marked reduction in ischemic stroke noted among patients in the warfarin treatment group observed in the BAATAF. (Stroke. 1993;24:1360-1365.)

KEY WORDS • anticoagulants • atrial fibrillation • clinical trials • warfarin

Seventy percent of the 400,000 strokes leading to acute hospitalization in the United States are due to ischemic cerebral vascular disease. Sixty percent of these are due to cerebral embolism from a cardiac source or unknown source.1-3 By far, the most common pathophysiological condition associated with cardiogenic embolism is atrial fibrillation. More than 60,000 strokes per year in the United States alone are related to this cardiac dysrhythmia.4

Recently, low-intensity warfarin anticoagulation (eg, international normalized ratio [INR] of 1.5 to 2.7) has been conclusively shown to be highly effective and safe in preventing ischemic stroke in patients with atrial fibrillation.5-8 Presumably, this powerful effect is due to inhibition of clot formation in the cardiac atria.

We wished to assess the effect of low-intensity warfarin anticoagulation on the actual level of activity of the hemostatic system. The prothrombin time test used to monitor the intensity of anticoagulation conferred by warfarin is not a direct measure of how much actual clotting is occurring in the hemostatic system in vivo. Rather, it is an indirect measure of the effect of warfarin on the synthesis of several procoagulant clotting factors. Recently, a radioimmunoassay for monitoring the plasma level of the prothrombin fragment F1+2 has been developed.9-12 This activation peptide is liberated when prothrombin is converted to thrombin and thereby serves as an in vivo marker of thrombin generation and the degree of activity of the hemostatic system. In this article we report the level of prothrombin fragment F1+2 in patients with atrial fibrillation sampled from the Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF), a trial assessing the effect of low-intensity warfarin therapy on prevention of stroke.

Subjects and Methods

Patient Population

F1+2 was measured in a sample of patients participating in the BAATAF. Patient selection and eligibility

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From the Neurology/Stroke Service and Medical Service at the Massachusetts General Hospital, the Medical Service at the Beth Israel Hospital, and Brockton-West Roxbury Department of Veterans Affairs Medical Center, Boston; Harvard Medical School, Boston; and the Department of Biology, Massachusetts Institute of Technology, Cambridge. A complete list of the participants in the Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF) appears in the "Appendix."
criteria for the BAATAF have been described previously. In brief, all patients with chronic sustained or intermittent atrial fibrillation who did not have mitral stenosis or left ventricular aneurysm or thrombus on two-dimensional echocardiography were eligible. Standard medical exclusionary criteria for anticoagulation therapy applied.

The patients described here are a subset of 125 BAATAF patients who were evaluated by the principal investigator at a routine follow-up. This was accomplished either at the coordinating center, in their home, or at one of the participating centers’ (see “Appendix”). All patients are categorized according to whether they were actually taking warfarin. The warfarin group includes six patients originally randomly assigned to the control group, and the no-warfarin control group includes two patients originally assigned to the warfarin group.

Blood Collection

Plasma was obtained from each patient for determination of prothrombin fragment F₁+₂ and fibrinopeptide A (FPA) using a two-syringe technique, as previously described. Briefly, venipunctures were performed atraumatically using 19-gauge scalp vein needles, and blood was drawn into plastic syringes preloaded with an anticoagulant cocktail containing a thrombin inhibitor, ethylenediaminetetraacetic acid, and aprotinin obtained from Byk-Sangtec, Dietzenbach, Germany; the ratio of anticoagulant to blood used was 0.1:0.9 (vol/vol). After collection of blood samples, plasma fractions were obtained by centrifugation at 4°C for 20 minutes at 3000 rpm and stored at −80°C before use for FPA and F₁+₂ assays. The total volume of whole blood obtained for each patient visit was approximately 10 mL.

Immunoassays

Plasma F₁+₂ concentrations were determined by double antibody radioimmunoassay as described in previous reports. The interassay variability of this assay is approximately 8%. The plasma levels of FPA were determined with radioimmunoassay kits purchased from Byk-Sangtec. The FPA levels were used as a means of assessing the quality of the venipunctures because traumatic needle sticks may result in markedly elevated FPA levels and artifactual elevation of the plasma F₁+₂ measurements (data not shown). Therefore, F₁+₂ data points for 9 patients (4 on warfarin) of an original group of 134 patients were discarded because plasma FPA was greater than or equal to 10 nmol/L. That left data from 125 patients available for analysis of F₁+₂. For reference, we reanalyzed the F₁+₂ data obtained from a healthy male population aged 65 to 75 years using the same antibody preparation as we used in the present study. The mean ±1 SD of F₁+₂ for those healthy males was 1.59 ± 0.14 nmol/L (n = 26) compared with our control patients of the same age (1.56 ± 0.10 nmol/L).

Prothrombin Time Measurements

Prothrombin time tests were done in multiple laboratories as part of the BAATAF monitoring protocol. INR values were not being reported by many of the laboratories used at the time of the BAATAF. Prothrombin times were not routinely performed at the same time that samples were collected for F₁+₂ and FPA determinations. Therefore, prothrombin time values reported herein were the chronologically closest to the measurement of F₁+₂ (54% of prothrombin times were done on the same day as the F₁+₂ sample; the remainder were done an average of 6 days from the F₁+₂ sample). Warfarin doses reported are the amount taken in the week before the prothrombin time.

Statistical Analysis

Given that the plasma levels of coagulation system markers in the various subsets of patients were usually found to be non-normally distributed, the Kruskal-Wallis one-way analysis of variance was used to test the difference between groups; subsequent pairwise comparisons were made using the Mann-Whitney U test. All tests presented are two-tailed.

Results

Sixty-two of the 125 patients were taking warfarin and 63 patients were not taking warfarin (Table 1). The mean age of both the warfarin and control groups was 69 years. Of those taking warfarin, the average dose per week was 28.9 mg (n = 58), and the average prothrombin time ratio was 1.35 (n = 59). Of the 63 patients not on warfarin, 33 (52%) of patients were taking chronic aspirin therapy (325 mg/d or more for 32 patients; 162.5 mg/d for the remaining patient). Of the numerous clinical features presented in Table 1, only two were significantly different in the two therapy groups. The control group had more patients with a history of prior myocardial infarction, and it also had a slightly smaller mean left atrial diameter.

The warfarin group had a 71% lower mean F₁+₂ level than the control group (Table 2). Warfarin treatment resulted in a marked shift in the distribution of F₁+₂ values toward the lower range (Figure). The effect was seen for both male and female patients. The slightly higher F₁+₂ values for female versus male control subjects is not statistically significant (P > 0.05). Although the number of female control subjects is small, the slightly higher trend in women may partly be due to their higher mean age (68 years for 46 men, 70.2 years for 17 women). F₁+₂ levels were higher among older patients (Table 3). This was true for both male and female patients. However, F₁+₂ levels were significantly lower in the warfarin treatment group at all ages. In fact, those patients in the warfarin group aged older than 75 years had a lower mean F₁+₂ than the youngest of patients (aged younger than 65 years) in the control group.

In contrast to warfarin, aspirin therapy appeared to have no consistent, significant effect on the F₁+₂ level (Table 4). This was true both for men and women and for each of the three age groups (not depicted in Table 4).

Discussion

Patients in the group treated with low-intensity warfarin had a 71% lower mean plasma level of the prothrombin fragment F₁+₂ than those not receiving warfarin. These results suggest a significantly lower level of in vivo activity of the hemostatic system. This effect was seen with both male and female patients. Consistent with reports from population studies, older patients in our sample had higher levels of F₁+₂. Nonetheless, in all age groups, the level of F₁+₂ was significantly lower in
the warfarin-treated patients compared with the control patients. The $F_{1+2}$ values for control patients taking aspirin were similar to those values for control patients not taking aspirin. This lack of effect of aspirin was similar for both men and women and at all age groups. It is also consistent with a large amount of unpublished data from our laboratory.

The positive effect of warfarin on the level of activity on the hemostatic system among these patients correlates well with the clinical results of the BAATAF. First, the significantly lower level of activity of the hemostatic system noted in patients taking warfarin correlates with the dramatic reduction in the incidence of stroke in the warfarin-treated BAATAF patients. Second, the age-related increase in $F_{1+2}$ among control patients also correlates with age being an important risk factor for stroke in BAATAF patients. This age-related increased incidence of stroke in patients with atrial fibrillation has also been seen in several large population-based studies, including the Framingham Study.14-17

The lack of effect of aspirin on the level of activity of the hemostatic system is of interest. Analyses of the effect of aspirin in the BAATAF, which did not benefit...
Bar graph shows distribution of plasma $F_{1+2}$ levels according to treatment subgroup (warfarin-treated vs non-warfarin-treated patients; n=62 and n=63, respectively).

from randomization, were consistent with a lack of stroke-preventive effect.\textsuperscript{5,18} Randomized assessments of the stroke-preventive effect of aspirin (in comparison to placebo) from two prospective studies (the Atrial Fibrillation, Aspirin, Anticoagulation [AFASAK] study and the Stroke Prevention in Atrial Fibrillation [SPAF] study) are inconsistent.\textsuperscript{6,7} In the AFASAK study,\textsuperscript{6} aspirin at 75 mg/d appeared to have minimal or no effect. In the SPAF study,\textsuperscript{7} aspirin at 325 mg/d was reported to have some effect. The effect was noted mainly in patients aged younger than 75 years who had minor strokes, many of whom had complete clearing of their symptoms. A subsequent publication from the SPAF study indicates that aspirin preferentially reduced the occurrence of strokes categorized as noncardioembolic as opposed to those categorized as cardioembolic.\textsuperscript{19}

The data presented in this article suggest but do not prove that $F_{1+2}$ is a marker of risk for cardioembolic stroke in atrial fibrillation. Our data directly provide an estimate of the physiological impact of low-intensity warfarin therapy. In a related study, we have shown that even lower “mini” intensity of warfarin therapy (target INR of 1.3 to 1.6) also produces a substantial lowering of $F_{1+2}$ levels,\textsuperscript{20} suggesting that very-low-intensity anticoagulation may confer protection against stroke in atrial fibrillation. We did not compare $F_{1+2}$ values in our control patients with age-matched normal subjects who were not fibrillating. However, we reanalyzed the data from a healthy male population aged 65 to 75 years\textsuperscript{13} using the same $F_{1+2}$ antibody preparation as was used in the present study and found that $F_{1+2}$ values were similar. This is in contrast to other work suggesting that the level of activity of the hemostatic system is increased in patients with atrial fibrillation as assessed by the concentration of D-dimer, the cross-linked fibrin derivative.\textsuperscript{21}

Our results demonstrate once again\textsuperscript{13} that the level of activity of the hemostatic system as measured by $F_{1+2}$ increases with age (here in patients with atrial fibrillation) and shows that this age-related effect is also dramatically reduced with low-intensity warfarin anticoagulation. Prospective measurement of $F_{1+2}$ in large samples of patients with atrial fibrillation is needed to further define its value in assessing stroke risk.

### Appendix

#### Participating Institutions and Investigators

The following institutions and investigators participated in the Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF). The numbers of patients enrolled at each center are given in parentheses. At group I institutions, on-site investigators performed all clinical evaluations. At group II and III institutions, neurologists and nurses from the coordinating center at the Massachusetts General Hospital scheduled and performed all evaluations; investigators from the coordinating center traveled to group II institutions, whereas patients from group III institutions were evaluated at the coordinating center.

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>n</th>
<th>$F_{1+2}$ (nmol/L)$\pm$1 SE</th>
<th>$F_{1+2}$ (nmol/L)$\pm$1 SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ Warfarin</td>
<td>− Warfarin</td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>38</td>
<td>0.32±0.06</td>
<td>1.26±0.12</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=22)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.06-1.18)</td>
<td>(0.47-2.10)</td>
<td></td>
</tr>
<tr>
<td>65-75</td>
<td>60</td>
<td>0.40±0.05</td>
<td>1.56±0.10</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=22)</td>
<td>(n=38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.08-0.89)</td>
<td>(0.51-3.15)</td>
<td></td>
</tr>
<tr>
<td>&gt;75</td>
<td>27</td>
<td>0.73±0.15</td>
<td>2.18±0.51</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=18)</td>
<td>(n=9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.18-2.54)</td>
<td>(0.87-4.59)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses indicate range.
TABLE 4. Effect of Aspirin on F_{1+2} Levels

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
<th>Mean age (y)</th>
<th>F_{1+2} (nmol/L)±1 SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aspirin</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>63</td>
<td>100</td>
<td>69.0±6.3</td>
<td>1.52±0.11</td>
<td>&gt;.10</td>
</tr>
<tr>
<td></td>
<td>(n=33)</td>
<td>1.64±0.18</td>
<td>(n=30)</td>
<td>(0.69-3.15)</td>
<td>(0.47-4.59)</td>
</tr>
<tr>
<td>Men</td>
<td>46</td>
<td>73</td>
<td>68.6±5.8</td>
<td>1.50±0.11</td>
<td>&gt;.10</td>
</tr>
<tr>
<td></td>
<td>(n=25)</td>
<td>1.29±0.11</td>
<td>(n=21)</td>
<td>(0.69-3.04)</td>
<td>(0.47-2.12)</td>
</tr>
<tr>
<td>Women</td>
<td>17</td>
<td>27</td>
<td>70.2±7.6</td>
<td>1.56±0.27</td>
<td>&gt;.05</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>2.45±0.46</td>
<td>(n=9)</td>
<td>(0.83-3.15)</td>
<td>(0.61-4.59)</td>
</tr>
</tbody>
</table>

Values in parentheses indicate range.

Group I (166 patients enrolled): Massachusetts General Hospital, Neurology and General Medicine Services, and Harvard Medical School, Boston (70): Daniel E. Singer, MD, Robert A. Hughes, MD, Daryl R. Gress, MD, Mary A. Sheehan, RN, Lynn B. Oertel, RN-C, MS, Sue Ward-Mauretano, RN, Dyan Ryan Blewett, MS, Bernard Rosner, PhD, and J. Philip Kirstler, MD; University of Utah, Salt Lake City (31): Gregory K. Call, MD, Jeffrey L. Anderson, MD, Thomas H. Caine, MD, Bruce Bray, MD, and Susan Lyver, RN; Boston City Hospital, Boston, Mass (17): Rodney H. Falk, MD, Nancy Battinelli, RN, G. Gargas, MD, Nagpoppel Venna, MD, Sheila Hewett, RN, and Michael McNeil, RN; University Hospital and Boston University, Boston, Mass (12): Michael D. Klein, MD, Philip A. Wolf, MD, Carlos S. Kase, MD, and Eloise E. Licata-Gehr, RN, MS; Penobscot Bay Medical Center, Rockland, Me (12): Donald J. Weaver, MD, Robert W. Stein, MD, Ralph Hamill, MD, and Patricia Cole, RN; University of Connecticut Health Center, Farmington (10): W. David Hager, MD, Hartwell G. Thompson, MD, and Denise Raymond, RN; Stanford University Medical Center, Palo Alto, Calif (9): Tim A. Fischell, MD, Richard L. Popp, MD, Gregory Albers, MD, Ann Cline, RN, and Pamela Galante, RN; University of Pittsburgh and Presbyterian Hospital, Pittsburgh, Pa (4): Lawrence R. Wechsler, MD, Richard Fogoros, MD, Nancy Kosanovich, RN-C, and Nancy Nagel, RN; Columbia-Presbyterian Medical Center, New York, NY (1): George Petty, MD.

Group II (210 patients enrolled): Dartmouth Hitchcock Medical Center—White River Junction Veterans Affairs Hospital, White River Junction, Vt (69): Arthur E. Sauvigne, MD, John Wasson, MD, Ann Choquette, and James Bell, MD; Mary Hitchcock Memorial Hospital, Hanover, NH (18): Andrew T. Torkelson, MD, John Plehn, MD, Alexander G. Reeves, MD, and Vera Deveau, RN; Brown University–Affiliated Hospitals: Providence Veterans Hospital, Providence, RI (18): Satish C. Sharma, MD, and Donna Wiberg, NP, RN-C; Miriam Veterans Hospital, Providence, RI (17): Ara Sadanianz, MD, William Stone, MD, Donna Fitzpatrick, RN, and Ann Nugent, RN; Memorial Hospital, Pawtucket, RI (14): Gary V. Heller, MD, Candace Miklozek-McNulty, MD, and Lisa Warren, MS; Rhode Island Hospital, Providence, RI (3): Jeffrey Austerlitz, MD, Jane Carter, MD, Nicole Aebersicher, MD, Satish C. Sharma, MD, and Joanne Austerlitz, RN; Mary Imogene Bassett Hospital, Cooperstown, NY (28): Lewis L. Hamilton, MD, Stephen E. Szebenyi, MD, David W. Vaules, MD, Herbert J. Marx, MD, Alan Kozak, MD, and Carol Bordley, RN; Mary Imogene Bassett Hospital–Herkimer Health Center, Herkimer, NY: Richard Trimble, MD, Philip Dwonczyk, MD, and Mark Darrow, MD; New Hampshire Heart Institute, Manchester (15): J. Beatty Hunter, MD, and Pamela Gagnon, RN; Lown Cardiovascular Group, Boston, Mass (7): Charles M. Blatt, MD, and Martha Constantino, RN; Malden Hospital, Malden, Mass (6):

Farouk A. Pirzada, MD, and Lawrence Moschitto, MD; Medical Center Hospital of Vermont–University of Vermont, Burlington (4): Mark A. Capeless, MD, and Jonathan Dissen, MD; Merrimack Valley Cardiology Associates, Chelmsford, Mass (3): Jose Carrion, MD, and Elizabeth Terranova, RN; Nashoba Community Hospital, Ayer, Mass (2): James Barzun, MD, and Gary Stanton, MD.

Group III (44 patients enrolled): Harvard Community Health Plan, Boston, Mass (18): Mark Stockman, MD; Newton-Wellesley Hospital, Newton, Mass (5): Mark R. Goldman, MD, Joel Rubenstein, MD, and Richard E. Toran, MD; Emerson Hospital, Concord, Mass (4): Charles S. Keevil, MD, and Michael Moore, MD; Brockton–West Roxbury Veterans Affairs Medical Center, Boston, Mass (3): William Strauss, MD; Cambridge Hospital, Cambridge, Mass (5): Thomas Rissler, MD, Salim Jabbour, MD, and Thomas Glick, MD, Jordan Hospital, Plymouth, Mass (3): Robert Timberlake, MD, and Lee I. Corwin, MD; Mount Auburn Hospital, Cambridge, Mass (2): Stanley A. Forwand, MD; Atlanticare, Lynn, Mass (1): Gerald M. Perlow, MD; private medical offices (3): Leslie Selbovitz, MD, Charles Sykes, MD, and Robert England, MD.

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