Monitoring "Mini-Intensity" Anticoagulation With Warfarin: Comparison of the Prothrombin Time Using a Sensitive Thromboplastin With Prothrombin Fragment F_{1+2} Levels

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Treatment with warfarin using a target International Normalized Ratio (INR) range of 1.7 to 2.5 is efficacious for many clinical indications, but the minimal intensity of anticoagulation required for antithrombotic protection has yet to be determined. To evaluate whether patients could be reliably monitored with a less intense regimen, we anticoagulated patients with warfarin for several months using a target INR range of 1.3 to 1.6 as determined by prothrombin time (PT) using a sensitive thromboplastin (Dade IS, International Sensitivity Index [ISI] = 1.3). Plasma measurements of F_{1+2}, a marker of factor Xa action on prothrombin in vivo, were also obtained to determine the suppressive effect of warfarin on hemostatic system activity. Overall, 20 of 21 patients with a history of cerebrovascular events (mean age, 61 years) could be reliably regulated with warfarin in the target INR range. F_{1+2} levels were significantly suppressed from baseline in all patients, with a mean reduction of 49% (range, 28% to 78%). We found a significant relationship between the extent of suppression of prothrombin activation levels and the baseline measurements. A mean reduction of 65% was observed for those patients with baseline $F_{1+2} \geq 1.5$ nmol/L, but only 38% for baseline $F_{1+2} \leq 0.5$ nmol/L. Overall, 68% of plasma samples obtained during stable anticoagulation were within the target INR range. PTs were also determined on all plasma samples with two thromboplastins of lower sensitivity (C+, ISI = 2.09; and automated simplastin, ISI = 2.10). Only 47% and 35% of PT determinations, respectively, were within the target range with these reagents. We conclude that prothrombin activation can be significantly suppressed in vivo with use of warfarin in an INR range of 1.3 to 1.6. This level of anticoagulation can be reliably achieved by monitoring PTs with a thromboplastin of high sensitivity.

THE USE OF low-dose warfarin (International Normalized Ratio [INR] = 1.7 to 2.5) has become standard for treating many clinical indications.[1-3] With increasing use of the drug for primary or secondary prophylaxis of thromboembolism,[4-10] it will be important to establish the minimum intensity of anticoagulation necessary for antithrombotic protection. In North America, warfarin therapy is monitored with the prothrombin time (PT) using thromboplastin reagents with an International Sensitivity Index (ISI) ≥ 2.0. To monitor warfarin therapy at minimal intensity (INR < 1.7), it may be advantageous to perform PT measurements with a relatively sensitive thromboplastin to provide a wider range (in seconds) in which to make fine adjustments in the dosage of warfarin.

Our laboratory has developed several specific and sensitive radioimmunoassays (RIAs) for monitoring plasma levels of the activation peptides of various coagulation proteins.[11-13] These serve as markers of the extent of hemostatic system activation in both normal patients and those with various inherited and acquired prethrombotic conditions.[14,15] We have previously shown the ability of very low-intensity warfarin therapy to suppress elevated plasma levels of fragment F_{1+2}, a marker of prothrombin activation in vivo, into the normal range in several patients with thrombotic diatheses.[16] The reduced measurements of F_{1+2} were not caused by alterations in the clearance or immunoreactivity of the fragment. In this small cohort of patients, the thromboplastin used to monitor PTs was relatively insensitive, with an ISI of approximately 2.0, and the PT ratios ranged between 1.0 and 1.2.

The current investigation was undertaken to determine whether patients could be reliably anticoagulated with adjusted "mini-intensity" warfarin using a more sensitive thromboplastin reagent (ISI = 1.3), and to correlate the INRs with changes in the plasma levels of F_{1+2}. We show that most patients can be stably anticoagulated with very low doses of warfarin, and that such regimens generally result in the suppression of baseline F_{1+2} levels by approximately 50%. These findings may have important implications for future trials of warfarin therapy at very low intensity for the prevention of thrombosis.

MATERIALS AND METHODS

Patient population. Participants in the study were referred by physicians of the Stroke Service at Massachusetts General Hospital. Individuals eligible for participation had either a history of cerebral embolism from an unknown source, or acute carotid occlusion resulting in a transient ischemic attack or stroke. All patients had either completed, or were in the process of completing, warfarin at standard intensity (INR 1.7 to 2.5) that had been prescribed by their physicians for secondary stroke prophylaxis.[17,18] Patients were excluded if there was any history of warfarin-related complications, including hemorrhage. A few persons with chronic nonrheumatic atrial fibrillation without a history of cerebrovascular events were also enrolled in the study. All individuals were outpatients for the duration of the study.

Study design. In patients who had not recently been treated with warfarin, baseline blood samples were obtained on two occasions at least 1 week apart, and they were then empirically

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begun on warfarin at doses ranging from 1 to 5 mg daily as guided by consideration of the individual aspects of the patient and/or previous dosage of medication (if available). Patients were then asked to return approximately every 2 weeks for PT measurements with subsequent adjustment of the warfarin dose to maintain the INR between 1.3 and 1.6. PTs were performed using a sensitive thromboplastin, Thromboplastin IS (Baxter Dade, Miami, FL; ISI = 1.3). Using this reagent, an INR range of 1.3 to 1.6 corresponds to a PT ratio of approximately 1.2 to 1.4. The INRs were calculated according to the following formula[19]: INR = (patient PT [sec]/normal pool PT [sec])^{ISI}. All patients had adjustments made in their warfarin dose until the "target dose" was achieved. This was defined as the amount of medication that resulted in an INR of 1.3 to 1.6 on two or more consecutive visits separated by an interval of at least 2 weeks. After a stable target dose was defined for each participant, PTs were monitored at 3- to 4-week intervals for a period of several months.

For those patients enrolled in the study while receiving warfarin at standard intensity, baseline blood samples were obtained on two occasions separated by at least 1 week to document stable anticoagulation. Patients subsequently had their warfarin doses reduced empirically into the "mini-intensity" range with follow-up PTs at approximately 2-week intervals. Dose adjustments were made as needed to maintain the INR between 1.3 and 1.6 using the sensitive thromboplastin as described above. After the period of stable anticoagulation at the target dose, patients were withdrawn from warfarin for at least 2 weeks. Blood samples were then obtained on one or two occasions to establish baseline measurements for the patient.

Collection and processing of blood samples. Venipunctures were performed atraumatically with 19- or 21-gauge butterfly infusion sets using a two-syringe technique. Blood samples for the F_{1+2} and fibrinopeptide A (FPA) RIAs were drawn into plastic syringes preloaded with an anticoagulant containing a thrombin inhibitor, EDTA, and aprotinin (purchased from Byk-Sangtec, Dietzenbach, Germany); the ratio of anticoagulant to blood used was 0.1:0.9 (vol/vol). After the collection of this sample, blood was drawn into a vacutainer (Becton Dickinson, Rutherford, NJ) containing 3.8% (wt/vol) sodium citrate; the ratio of anticoagulant to blood was 0.1:0.9 (vol/vol). Plasma fractions were obtained by centrifugation at 4°C for 20 minutes at 1,600g and samples for the F_{1+2} and FPA immunoassays were stored at −80°C before use.

Coagulation studies. PTs were performed on fresh samples of citrated plasma using a MLA Electra 900C (Medical Laboratory Automation, Inc, Pleasantville, NY). In addition to using Thromboplastin IS, the PTs of plasmas were also determined with this instrument and a less sensitive thromboplastin reagent, Thromboplastin C+ (Baxter Dade; ISI = 2.09), that is frequently used in clinical laboratories in North America. In addition, PTs were performed on a Coag-a-mate X-2 (General Diagnostics, Morris Plains, NJ) using Automated Simplastin (Organon Teknika Corp, Durham, NC; ISI = 2.1) as the thromboplastin reagent.

Immunoassays. The plasma concentrations of F_{1+2} were determined by double antibody RIAs as described in previous reports from our laboratory.[11,12] The interassay variability of this assay is approximately 8%. The plasma levels of FPA were established by RIA using kits purchased from Byk-Sangtec. The FPA levels were used to assess the quality of the venipunctures because traumatic needle sticks usually result in markedly increased FPA levels and artifactual elevation of plasma F_{1+2} measurements (unpublished data). We therefore excluded F_{1+2} data points from our analysis if the FPA level on a given plasma was ≥ 10 nmol/L.

Informed consent. Informed consent was obtained from all participants. This study was approved by the Committee on Clinical Investigations, New Procedures and New Forms of Therapy of Beth Israel Hospital, and the Subcommittee on Human Studies of Massachusetts General Hospital.

Analysis of data. Statistical analyses of data were conducted using standard techniques.[20] The ΔF_{1+2} results were calculated according to the formula:

$$\Delta F_{1+2} = ([\text{measured } F_{1+2} - \text{baseline } F_{1+2}]/\text{baseline } F_{1+2}) \times 100$$

The relationship between ΔF_{1+2} and INR were analyzed by linear regression with line fitting, using the method of Deming as previously described.[21] The correlations between INRs obtained with different thromboplastins were determined by linear regression analysis using the method of least squares.

RESULTS

A total of 22 patients were enrolled in the study over a period of 9 months. One patient was excluded shortly after enrolling to undergo elective coronary revascularization. Of the remaining 21 patients, there were 14 men and 7 women, with a mean age of 61 years (range, 36 to 86). Thirteen patients (62%) had a history of embolic stroke from an unknown source, six (29%) had carotid occlusion, and two (9%) had chronic atrial fibrillation without a history of stroke. At the time of enrollment, 11 patients were not receiving warfarin, while 10 patients were stably anticoagulated at standard intensity (INR 1.7 to 2.5). The mean duration of follow-up after starting on "mini-intensity" warfarin was 26.3 weeks (range, 9 to 43 weeks).

Overall, 20 of the 21 participants (95%) achieved stable anticoagulation on mini-intensity warfarin within the target INR range of 1.3 to 1.6 using the sensitive thromboplastin reagent (ISI = 1.3). The one patient who did not achieve a stable anticoagulant effect was a 57-year-old woman who was not receiving other medications and reported excellent warfarin compliance. The results obtained in the remaining 20 patients are summarized in Table 1. The mean weekly warfarin dose for this group was 26.0 mg (range, 12.5 to 52.5 mg; or mean daily dose, 3.7 mg). Three patients were taking medications known to decrease the bioavailability of warfarin (mysoline, tegretol, and cholestyramine) and required considerably higher doses of warfarin (mean daily dose, 5.5 mg) than the remainder of the patient population. If these patients are excluded, the mean weekly warfarin dose was 22.6 mg (range, 12.5 to 35.0 mg; or mean daily dose, 3.2 mg). The patients required a mean of 3.9 weeks and 2.3 PT measurements to achieve stable anticoagulation in the "mini-intensity" range.

A total of 256 samples were obtained for simultaneous determinations of PT and F_{1+2} levels in all 21 patients (mean, 12.2/patient). Sixty-one blood samples were drawn for baseline F_{1+2} determinations in the absence of warfarin therapy (mean, 2.9/patient). Among the 256 samples, five data points were excluded from analysis (three resulted from unsatisfactory venipunctures as documented by FPA ≥ 10 nmol/L, one from admitted noncompliance with warfarin, and one was inadvertently awn within 1 week after adjusting the warfarin dose). Among the remaining 190 samples, 82 were obtained while patients were having their warfarin dose adjusted and 108 were taken while

Table 1. Plasma F_{1+2} Levels in Patients on "Mini-Intensity" Warfarin

Subject	Age	Baseline F_{1+2}	Target F_{1+2}	% Decrease	Dose*
1	53	0.29	0.21	27.6	35.0
2	53	0.72	0.36	50.0	32.0
3	55	0.96	0.25	74.0	44.0†
4	54	0.77	0.40	48.1	28.0
5	71	1.24	0.63	49.2	14.0
6	86	4.90	1.10	77.6	12.5
7	68	0.71	0.41	42.2	14.0
8	51	0.43	0.24	44.2	52.5†
9	73	1.47	0.69	53.1	17.5
10	68	0.50	0.28	44.0	31.5
11	81	0.63	0.35	44.4	14.0
12	68	0.65	0.42	35.4	14.0
13	72	1.93	0.70	63.7	28.0
14	36	0.81	0.42	48.1	40.0†
15	69	0.59	0.33	44.1	35.0
16	42	0.23	0.15	34.8	28.0
17	50	1.59	0.90	43.4	28.0
18	66	1.01	0.78	22.8	14.0
19	39	2.01	0.53	73.6	24.0
20	69	0.53	0.24	54.7	14.0
Mean ± SD		1.10 ± 1.03‡	0.47 ± 0.25	48.8 ± 14.2	26.0 ± 11.4

*Doses are reported as milligrams per week.

†Patients on medications known to decrease the bioavailability of warfarin.

‡With the preparation of F_{1+2} antibody used for this study, the mean plasma level of F_{1+2} in a control group of healthy age-matched individuals was 0.85 ± 0.30 nmol/L.

patients were on their target dose (mean, 5.35/patient). A total of 73 of 108 samples (67.6%) had PTs in an INR range of 1.3 to 1.6 using the sensitive thromboplastin reagent. In these 108 plasma samples, the INRs as determined by PT, using the less sensitive thromboplastin reagents, were less often within the 1.3 to 1.6 range (51 of 108 [47.2%] for the Thromboplastin C+ reagent and 38 of 108 [35.2%] for the automated simplastin reagent).

We then analyzed the suppression of plasma F_{1+2} levels (expressed as percent decrease from baseline) in the 20 patients achieving stable anticoagulation on "mini-intensity" warfarin (Table 1). The mean baseline F_{1+2} concentration in the group was 1.10 ± 1.03 nmol/L, while the mean level was 0.47 ± 0.25 nmol/L in patients who were stably anticoagulated at their target warfarin dose ($P < .005$). This represents a mean reduction of 48.8% in baseline F_{1+2} measurements. We found a significant relationship between the extent of suppression of F_{1+2} levels and the baseline measurements ($r = -.66$, $P < .0001$). For example, the mean reduction in F_{1+2} for those patients with baseline levels ≥ 1.50 nmol/L was 64.6% (range, 43.4 to 77.6; n = 4), while that for patients with baseline levels ≤0.5 nmol/L was only 37.6% (range, 27.6 to 44.2; n = 4). Patients with baseline F_{1+2} concentrations between 0.5 and 1.5 nmol/L had a mean reduction of 47.2% (range, 22.8 to 74.0; n = 12). No correlation was found between the target dose of warfarin and the extent of reduction in F_{1+2} measurements (Table 1). This emphasizes the heterogeneity among individuals with regard to the suppression of prothrombin activation in response to warfarin. There was a weak inverse correlation between age and target warfarin dose; among patients over the age of 65, 8 of 11 (73%) required less than 18 mg weekly, while among those less than 65, 6 of 9 (67%) required ≥24 mg weekly.

In patients achieving stable anticoagulation on "mini-intensity" warfarin, we retrospectively analyzed our data by defining a target range for the extent of suppression of baseline F_{1+2} levels. This was arbitrarily taken to be 30% to 70%, and we found that 86 of 108 plasmas (79.6%) had F_{1+2} measurements within this range. This is similar to the percentage of plasmas with PTs in the target INR range using the sensitive thromboplastin reagent. We then analyzed the correlation of Δ F_{1+2} versus INR using the thromboplastin IS reagent for all 190 plasma samples (Fig 1). Although there is considerable scatter of the data points, the r value of −.47 is highly significant ($P < .0001$). The correlations of Δ F_{1+2} versus INR for the other two thromboplastin reagents give similar r values that are also highly significant (data not shown).

We also calculated the percentage of PTs that were within the normal range for each thromboplastin reagent (defined as the mean obtained on individual normal pool plasmas ± 2 SD) while patients were on their target warfarin dose. We found that only 0.9% of the target dose PTs using the IS reagent fell within the normal range, as compared with 14.8% for the C+ reagent and 39.8% for the automated simplastin reagent (Table 2). These data strongly suggest that a sensitive PT reagent is most efficacious in monitoring "mini-intensity" warfarin therapy.

The INR results for the three different thromboplastin reagents were also compared by linear regression analysis.

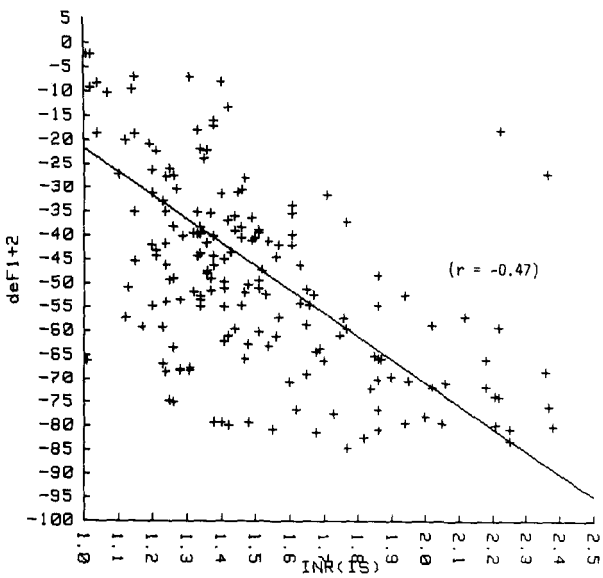

Fig 1. Plot of INR using Thromboplastin IS (ISI = 1.3) versus delta (de) F_{1+2} ([(measured F_{1+2} − baseline F_{1+2}}/baseline F_{1+2}] × 100) r = −.47.

Table 2. Comparison of PT Measurements With Different Thromboplastin Reagents in Patients on "Mini-Intensity" Warfarin

Reagent	ISI	Mean (PT) (s)*	PT Range (s)†	No. in Normal Range (%)
Thromboplastin IS	1.30	13.8	12.2-15.5	1/108 (0.9)
Thromboplastin C+	2.09	12.0	11.0-13.0	16/108 (14.8)
Automated simplastin	2.10	10.1	9.2-11.0	43/108 (39.8)

*Derived from a normal plasma pool of greater than 50 donors.

†Defined as the mean ± 2 SD for PT measurements performed on individual plasmas of donors to normal pool.

The correlation coefficients between the various reagents were excellent with r values of .92 to .96. However, the slopes were significantly different from unity, (0.8 for IS v automated simplastin, 0.79 for IS v C+, and 0.95 for C+ v automated simplastin), and the thromboplastin IS reagent gave slightly higher INR values than the two less sensitive reagents.

DISCUSSION

The use of oral anticoagulants at very low intensity represents an attractive therapeutic modality in patients requiring long-term thrombosis prophylaxis. If such a regimen were proven to be efficacious, it seems likely that it would be associated with a lower bleeding risk than warfarin at standard intensity, and might also require less frequent laboratory monitoring. One approach that has been used to achieve oral anticoagulation at very low intensity is the administration of small fixed doses of warfarin (1 to 2 mg daily), which in most patients has little effect on the prothrombin time.[22,23] Although such a schedule is simple for the patient and the clinician, it is not pharmacologically appropriate, given the tremendous heterogeneity in warfarin bioavailability and response between individuals. It could be anticipated that many patients receiving small fixed-dose regimens would show little or no suppression of coagulation system function. A more rational approach would be to administer adjusted doses of warfarin with the goal of maintaining the INR below 1.7 to 2.5, which corresponds to the range that has thus far been shown to be efficacious for a variety of clinical indications.

To evaluate whether patients could be reliably anticoagulated with adjusted "mini-intensity" warfarin, we monitored PTs for several months in patients who were prone to developing thromboembolic cerebrovascular events. PTs were determined using a sensitive thromboplastin reagent and the target INR range was 1.3 to 1.6. Our results demonstrated that 20 of 21 patients could be stably anticoagulated with very low doses of warfarin using this monitoring strategy, and that such a regimen suppressed F_{1+2} levels by approximately 50% from baseline. In the one patient who could not be stably anticoagulated, we speculate that day to day fluctuations in dietary vitamin K intake may have been responsible for the lability of the PT measurements.

We also found that the thromboplastin with an ISI of 1.3 was superior to the less sensitive reagents in monitoring adjusted "mini-intensity" warfarin. After patients were stably anticoagulated in an INR range of 1.3 to 1.6, follow-up measurements were usually within this target interval. In contrast, PTs performed with the less sensitive reagents often indicated that the anticoagulant effect was subtherapeutic. It seems likely that a monitoring strategy based on INR measurements obtained with one of the less sensitive thromboplastins would have led to the use of higher doses of warfarin.

A critical issue that has not been addressed in the present study is the minimum degree of suppression of prothrombin activation required to provide antithrombotic protection in a given clinical setting. Suppression of vitamin K-dependent factor levels to approximately 50% of normal has been shown to be effective in preventing the development of stasis thrombi in animal models.[24,25] In humans, stable anticoagulation with warfarin in an INR range of 1.7 to 2.5 results in vitamin K-dependent factor levels of approximately 30% of normal.[26-28] As the F_{1+2} level is a measure of prothrombin activation resulting from the balance between prothrombotic and antithrombotic forces in vivo, it may be a more relevant parameter with which to monitor warfarin therapy as compared with PT determinations or vitamin K-dependent factor levels.

We have retrospectively analyzed the extent of prothrombin activation in the blood of 130 patients who were enrolled in the Boston Area Anticoagulation Trial for Atrial Fibrillation (BAATAF) in which warfarin was administered to patients with nonrheumatic atrial fibrillation using an INR range of 1.7 to 2.5.[2] This clinical study demonstrated an 86% reduction in the incidence of embolic stroke in patients receiving oral anticoagulants, and we observed that plasma F_{1+2} measurements in treated patients were decreased by approximately 70% as compared with untreated controls (unpublished data). Based on the clinical results of the BAATAF trial and the results of this investigation, we believe that adjusted "mini-intensity" warfarin is an appropriate approach for using this drug in future clinical studies designed to determine the minimal intensity of medication that is required for antithrombotic efficacy.

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