Monitoring “Mini-Intensity” Anticoagulation With Warfarin: Comparison of the Prothrombin Time Using a Sensitive Thromboplastin With Prothrombin Fragment Fₙ₂ Levels

By Michael M. Millenson, Kenneth A. Bauer, J. Philip Kistler, Samad Barzegar, Lila Tulin, and Robert D. Rosenberg

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 Fₙ₂, 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 anticoagulated with warfarin in the target INR range. Fₙ₂ 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 Fₙ₂ ≥ 1.5 nmol/L, but only 38% for baseline Fₙ₂ ≤ 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.

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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: \[ \text{INR} = \frac{\text{patient PT (sec)/normal pool PT (sec)}}{\text{ISI}} \]

The relationship between \( \Delta \text{F}_{\text{I}+2} \) and INR was analyzed by linear regression with line fitting, using the method of Deming as previously described. 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 (misonidazole, 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 \( \text{F}_{\text{I}+2} \) levels in all 21 patients (mean, 12.2/patient). Sixty-one blood samples were drawn for baseline \( \text{F}_{\text{I}+2} \) 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 measurements taken at unsatisfactory venipunctures as documented by FPA measurements (unpublished data). We therefore excluded \( \text{F}_{\text{I}+2} \) data points from our analysis if the FPA level on a given plasma was \( \geq 10 \text{ nmol/L} \).
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\textsubscript{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\textsubscript{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\textsubscript{1+2} measurements. We found a significant relationship between the extent of suppression of F\textsubscript{1+2} levels and the baseline measurements (r = -.66, P < .0001). For example, the mean reduction in F\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{1+2} levels. This was arbitrarily taken to be 30% to 70%, and we found that 86 of 108 plasmas (79.6%) had F\textsubscript{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\textsubscript{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\textsubscript{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.

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

![Fig 1. Plot of INR using Thromboplastin IS (ISI = 1.3) versus delta (de) F\textsubscript{1+2} ([measured F\textsubscript{1+2} − baseline F\textsubscript{1+2}] / baseline F\textsubscript{1+2} × 100) r = −.47.](image-url)
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+ v, and 0.95 for C+ v automated simplastin), and the thromboplatin 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. 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. 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. 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. 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.

**ACKNOWLEDGMENT**

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**REFERENCES**


11. Lau HK, Rosenberg JS, Beeler DL, Rosenberg RD: The isolation and characterization of a specific antibody population directed against the prothrombin activation fragments F2 and F1+2. J Biol Chem 254:8751, 1979


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