Rapid Communication

Effect of Treatment With Low-Dose Warfarin-Aspirin on Activated Factor VII

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Factor VII is an independent risk factor for ischemic heart disease. We performed a prospective study to evaluate the effect of combined low-dose warfarin-aspirin on activated factor VII (factor VIIa) and to determine if abruptly stopping this treatment is associated with a rebound in the level of factor VIIa. Thirty-three patients with clinically stable coronary artery disease were treated with combined 3 mg warfarin and 80 mg aspirin daily for 8 weeks. The factor VIIa level was measured before treatment, weekly during treatment, and 2 weeks after stopping treatment. The mean percent of pretreatment levels of factor VIIa for weeks 1 through 8 of treatment were 60%, 60%, 72%, 70%, 71%, 70%, 74%, and 87%, respectively (P < .05 compared with pretreatment for weeks 1 through 7 inclusive); 2 weeks after stopping treatment, the level was 122% (95% confidence interval [CI] 111% to 133%); P < .001 compared with pretreatment). The mean percent level of factor VIIa on-treatment was 74% (P < .001). Factor VIIa is reduced by 26% on average during treatment. This finding provides further rationale for the antithrombotic effect of low-dose warfarin. The results suggest a rebound in the factor VIIa level may occur after treatment is stopped. The potential rebound and its clinical importance should be evaluated by further studies.

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O R A L A N T I C O A G U L A N T T H E R A P Y with warfarin sodium is effective antithrombotic treatment for several indications.1,12 The therapeutic range for warfarin treatment has been revised downward in recent years because clinical trials indicate less intense therapy is safer while retaining antithrombotic efficacy.13,17 However, the lower limit of the therapeutic range has not been established for any indication and the minimum intensity of warfarin anticoagulant effect required for the treatment of thromboembolism is uncertain. Low doses of warfarin that produce little or no prolongation of the prothrombin time have antithrombotic efficacy in two clinical settings: (1) the prevention of catheter-related thrombosis in patients with chronic indwelling central venous catheters,14 and (2) the prevention of postoperative venous thrombosis in patients undergoing major gynecologic surgery.15 These observations, and the desire to simplify warfarin therapy by decreasing the need for laboratory monitoring, have stimulated interest in the use of fixed low doses for long-term antithrombotic treatment. Low-dose warfarin is well suited for combined use with aspirin in the prevention and treatment of arterial thromboembolic disease.3,20

This article, "low-dose warfarin" refers to fixed doses of 3 mg per day or lower, and "less intense" refers to warfarin adjusted to maintain the International Normalized Ratio (INR) between 2.0 and 3.0.13,17

The antithrombotic effect of warfarin is usually attributed to inhibition of the synthesis of functional vitamin K–dependent coagulation factors II, VII, IX, and X.1,4,17,23,24 For one of these, factor VII, elevated coagulant activity (VII:C) is independently associated with an increased risk of ischemic heart disease and mortality in middle-aged men.35 Factor VII exists in plasma both as the inactive form (zymogen) and the enzymatically active form (factor VIIa).26,27 The VII:C assay measures an aggregate of both zymogen factor VII and factor VIIa. Recently, a new assay that enables direct measurement of factor VIIa in plasma has been developed.17 This assay is specific for factor VIIa and free of interference by zymogen factor VII.27 The effect of low-dose warfarin on factor VIIa levels per se has not been previously evaluated.

Several authors have suggested abrupt cessation of oral anticoagulant therapy produces a rebound hypercoagulable state.28,33 This concept has been controversial29–34 since it was first suggested 40 years ago.29,30 Reports of thromboembolic events clustering early after abruptly stopping oral anticoagulant therapy29,30,32,34,35,41,42 have been used to support the concept of a rebound hypercoagulable state. However, it is unclear if these thromboembolic results eventuate from a true rebound hypercoagulability, or from the ongoing underlying disease which is unmasked when anticoagulant therapy is withdrawn.24 A true rebound phenomenon, in which a vitamin K–dependent procoagulant returns to a level higher than before treatment, has never been conclusively shown.

We performed a prospective study to evaluate the effect of combined low-dose warfarin-aspirin treatment on the levels of factor VIIa, and to determine if abruptly stopping this treatment is associated with a rebound in the level of factor VIIa.

MATERIALS AND METHODS

The study was performed in 33 patients with clinically stable coronary artery disease who gave informed consent and who were

3034

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enrolled in the Coumadin Aspirin Reinfarction (CARS) pilot study. All patients enrolled in the CARS pilot study at the University of Oklahoma (Oklahoma City, OK) were included in the present study. The study was performed between mid-September and the end of December 1992. The study protocol was approved by the institutional review board.

Patients. The patients were identified through the medicine clinics at University Hospital and Veterans Administration Medical Center of Oklahoma City. Patients were eligible if they were between 50 and 80 years of age and were clinically stable, and had a history of coronary artery disease defined by the presence of one or more of the following: a history of myocardial infarction documented objectively, previous coronary angioplasty, coronary artery bypass surgery more than 2 months previously, a positive exercise test or gated left ventricular blood pool scan within the past 12 months, or a coronary angiogram showing greater than 70% narrowing in one or more major coronary artery.

Patients were ineligible if they had one or more of the following: a requirement for treatment with anticoagulants; a history of hyper-sensitivity to warfarin or aspirin; current unstable angina; a history of cerebrovascular accident, documented peptic ulcer disease or gastrointestinal bleeding within the past 12 months; or congestive heart failure (New York Heart Association Class III or IV). Patients were also ineligible if the pretreatment laboratory evaluation identified one or more of the following: International Normalized Ratio greater than 1.3 or partial thromboplastin time above the upper limit of normal; hemoglobin less than 11 g/dL; transaminase enzymes, alkaline phosphatase, or bilirubin more than twice the upper limit of normal; creatinine greater than 2.5 mg/dL; or blood urea nitrogen greater than 50 mg/dL, or abnormal thyroid profile.

Study protocol. Before treatment, all patients had a clinical history taken, a physical examination performed and a venous blood sample obtained for measurement of the prothrombin time and the level of factor VIIa. All patients then began combined treatment with warfarin (Coumadin) in a dose of 3 mg daily and aspirin in a dose of 80 mg daily.

All patients were seen in the clinic on the last day of weeks 1 through 8 from the start of treatment. At each visit, the patients had an interval history taken addressing general health and adverse effects of treatment. A venous blood sample was obtained for measurement of the prothrombin time and factor VIIa level. The clinic visits were scheduled at similar times in the morning for each patient to minimize potential variation in the factor VIIa level caused by the time of day samples were obtained.

Treatment was continued for 8 weeks and then stopped abruptly without tapering. Treatment was discontinued earlier if the patient developed clinically evident bleeding or other adverse effects, or if the prothrombin time, expressed as the INR, was 4.5 or more at any time or if the INR remained 3.0 or more on two consecutive evaluations despite reducing the warfarin dose to 1 mg daily. All patients were seen in the clinic 2 weeks after completing each treatment (ie, week 10), and were evaluated as described above. Compliance was assessed by questioning the patients about their medication habits at each visit and by performing a pill count. The detailed methods and results of the CARS pilot study are reported elsewhere.

Blood collection and processing. Venipuncture was performed using a 21-gauge needle. Blood was collected directly into siliconized glass vacuum collection tubes containing buffered sodium citrate (Becton Dickinson Vacutainer Systems, Rutherford, NJ). A 2-tube collection technique was used. Only the second tube was used for measurement of factor VIIa. Platelet-poor plasma was prepared from the citrated blood by centrifugation at 3,200g for 20 minutes.

All processing of blood and plasma was at room temperature using polystyrene or polypropylene centrifuge tubes and pipets. Plasma samples were stored frozen at −70°C in plastic containers. Laboratory quality-control studies had shown that storage of plasma under these conditions for 9 months did not result in a detectable decrease in factor VIIa activity. The prothrombin times and factor VIIa assays were performed as one batch on all plasma samples obtained during the study. The frozen plasma samples were thawed rapidly at 37°C just before assay and kept at room temperature until assayed.

Measurement of the prothrombin time and factor VIIa. The prothrombin time and the factor VIIa assay were performed on aliquots of the same plasma sample. Both tests were performed using a coagulometer model ACL 300+ (Instrumentation Laboratories, Lexington, MA). The factor VIIa levels and the prothrombin times were measured without knowledge of each other. Both tests were performed on coded samples without knowledge of the date the sample was collected, the patient’s name, and whether the patient was on or off treatment with warfarin-aspirin.

The prothrombin time was measured using Simplastin Exel thromboplastin reagent with an International Sensitivity Index of 2.01 (Organon Teknika, Durham, NC). The mean prothrombin time on a pool of plasma from normal donors was 10.2 seconds. The prothrombin time results were expressed as the INR.

Factor VIIa levels were measured using an automated soluble tissue factor clotting assay described in detail previously.

Statistical analysis. The mean INR values and mean factor VIIa levels, and the 95% confidence intervals [CI] for these means, were calculated using the SAS statistical software package (SAS Institute, Cary, NC). The data on mean INR values and mean factor VIIa levels were analyzed using a repeated measures analysis of variance followed by a Newman-Keuls multiple comparison procedure. The data were also analyzed using the method of summary measures described by Matthews et al. For this summary measures analysis, the mean on-treatment values for each patient were compared with their pretreatment value, and the posttreatment values were compared with the pretreatment value. These paired comparisons were analyzed by the paired t-test, and also using the Wilcoxon matched-pairs signed-rank test.

RESULTS

Patients. The 33 patients ranged in age from 51 to 78 years (mean 64); 30 were male and 3 were female. The clinical characteristics of the patients are shown in Table 1.

Adherence to protocol. The 8-week treatment protocol was completed in 27 (82%) of the 33 patients; in six patients, treatment was discontinued before the protocol was completed. The combined warfarin-aspirin treatment was discontinued early in two patients because of clinically evident minor bleeding [one had intermittent self-limiting epistaxis during the third week (INR, 1.25) and the other had minor rectal bleeding during the seventh week (INR, 1.77)]. Three additional patients reported nonhemorrhagic adverse symptoms and discontinued the study treatment early. The remaining patient had treatment discontinued at the end of the first week because of a very prolonged INR (9.49) without clinically evident bleeding.

The pill count in the 27 patients who completed the treatment indicated 6 patients (22%) returned none of the prescribed medication, 20 patients (74%) returned 5% or less of the prescribed medication, and in one patient, 12% of the prescribed medication was returned.

Prothrombin time and factor VIIa. The INR values before treatment ranged from 0.85 to 1.11 (mean, 0.98; 95% CI; 0.96 to 1.00). The factor VIIa levels before treatment
The INR became prolonged to more than 3.0 in 7 of the 33 patients (21%; 95% CI: 9.0% to 38.9%). This occurred at week 1 in 2 patients, at week 2 in 3 patients, at week 4 in 1 patient and at week 6 in the remaining patient. The INR became prolonged to more than 4.5 in one patient and this patient had warfarin discontinued. In the remaining six patients, the INR was prolonged above 3.0, but less than 4.5. These patients had the warfarin dose reduced to 1 mg daily and completed the treatment protocol.

The mean INR values and the mean factor VIIa levels during the 8 weeks of treatment with warfarin 3 mg and aspirin 80 mg are shown in Fig 1, with the corresponding 95% CI. The mean INR and mean factor VIIa levels before treatment and 2 weeks after stopping treatment (week 10) are also shown for comparison. The mean on-treatment INR was 1.58 (P = .0002 compared with pretreatment by the paired t-test; \( P < .001 \) by the Wilcoxon matched-pairs signed-rank test). The mean on-treatment factor VIIa level was 2.99 ng/mL (\( P = .0006 \) compared with pretreatment by the paired t-test; \( P = .0008 \) by the Wilcoxon matched-pairs signed-rank test).

The factor VIIa levels were also expressed as a percentage of the patient’s pretreatment value. The mean percent levels of factor VIIa are shown in Fig 2, with the corresponding 95% CI. The mean percent levels of factor VIIa during treatment with warfarin 3 mg and aspirin 80 mg on weeks 1 through 8 respectively were 60%, 60%, 72%, 70%, 71%, 70%, 74%, and 87%. The results for weeks 1 through 7 inclusive are statistically significantly lower than pretreatment (\( P < .05 \)). The mean percent level of factor VIIa on-treatment was 74% (\( P = .0002 \) compared with pretreatment by the paired t-test; \( P = .0008 \) by the Wilcoxon matched-pairs signed-rank test).

The possibility of a rebound in the level of factor VIIa was evaluated by comparing the level 2 weeks after stopping treatment (week 10) with the pretreatment levels. This analysis was done in 26 patients who completed the treatment protocol; one additional patient who completed the treatment protocol did not have a week 10 sample obtained. The mean factor VIIa level 2 weeks after stopping treatment was 4.96 ng/mL, compared with the pretreatment value of 4.14 ng/mL (\( P = .0005 \) by the paired t-test; \( P = .0007 \) by the Wilcoxon matched-pairs signed-rank test).
LOW-DOSE WARFARIN AND FACTOR VII

Fig 3. Relation between the INR and the level of factor VIIa. The INR and corresponding level of factor VIIa (ng/ml) were determined on aliquots of the same plasma sample. Each point may represent more than one plasma sample.

The level of factor VIIa after stopping treatment for each patient was also expressed as a percentage of their pretreatment level. The mean percent level of factor VIIa 2 weeks after stopping treatment was 122% (95% CI; 111% to 133%; \( P = .0007 \) compared with pretreatment by the paired \( t \)-test; \( P = .0005 \) by the Wilcoxon matched-pairs signed-rank test). Of the 26 patients, the factor VIIa level ranged from 100 to 110% of their pretreatment level in 6 patients, 111% to 120% in 7 patients, 121% to 130% in 2 patients, 131% to 140% in 2 patients, 141% to 150% in 2 patients, and in four patients, the factor VIIa level was more than 150% of pretreatment. In three patients, the level of factor VIIa at the tenth week was less than the pretreatment level (65%, 86%, and 90% respectively).

The relation between the INR and factor VIIa level for all plasma samples collected during the study is shown in Fig 3.

DISCUSSION

The results suggest two inferences. First, the level of factor VIIa is reduced by 26% on average during combined treatment with fixed low-dose warfarin (3 mg) and aspirin (80 mg). This finding provides further rationale for the antithrombotic effect of low-dose warfarin. Second, the results suggest a rebound in the level of factor VIIa may occur after treatment is stopped. The factor VIIa level was an average of 22% higher 2 weeks after stopping treatment than before treatment (95% CI; 11% to 33%).

Before accepting these inferences, it is important to consider if the results can be explained by bias. In this study, the percent change in factor VIIa was assessed by comparing the level during and after treatment with the patient’s own pretreatment level. The patient is their own control in this analysis. Because a concurrent untreated control group was not included, we must consider the possibility that the observed changes in factor VIIa are caused by variation with time and may be unrelated to the treatment given. The factor VIIa level was decreased by 40% one week after treatment was started, and was increased 2 weeks after treatment was withdrawn (Fig 2). The timing of these changes in factor VIIa in relation to giving and withdrawing treatment suggests the changes are the result of treatment rather than variation in the patients. The possibility of rebound was assessed by comparing posttreatment levels with the pretreatment levels 10 weeks earlier. It is possible that variation in factor VIIa levels may have occurred over this time that is unrelated to treatment with warfarin-aspirin. The design of the present study cannot exclude this possibility. Our findings provide further support for the hypothesis that rebound occurs. A randomized controlled study should be performed to definitively resolve this hypothesis.

We evaluated the effect of low-dose warfarin-aspirin treatment on factor VIIa because factor VII coagulant activity is an independent risk factor for ischemic heart disease. In the Northwick Park Heart Study, the incidences of myocardial infarction and death from ischemic heart disease were three times and eight times higher, respectively, among patients in whom factor VII coagulant activity was 119% or more, compared with those in whom it was 98% or less. Although factor VII coagulant activity and factor VIIa may not be directly comparable, it is of interest that the changes in factor VIIa produced by low-dose warfarin-aspirin are similar in magnitude to the differences in factor VII coagulant activity that separate patients into markedly different risk groups for myocardial infarction and death from ischemic heart disease. Arguing against the importance of factor VII is the recent data of Zivelin et al from an experimental animal model of tissue-factor–induced coagulation. These investigators showed that immunodepletion of factor VII did not protect normal rabbits against tissue-factor–induced coagulation, whereas immunodepletion of prothrombin and factor X did. The clinical importance of these experimental animal findings is uncertain. Moreover, Millenson et al have shown that treatment with warfarin adjusted to maintain the INR between 1.3 and 1.6 resulted in marked suppression of prothrombin activation, as shown by an average reduction in prothrombin fragment F1.2 levels of 49% (range, 28% to 78%). The anticoagulant intensity (INR, 1.3 to 1.6), which achieved this effect is similar to that observed in our study (Fig 1). The mean INR of 1.58 achieved in our study is similar to the target INR in the Northwick Park primary prevention trial. Our findings and those of Millenson et al provide a biologic rationale for the antithrombotic effect of low-dose warfarin. It is possible that aspirin may have potentiated the effect of warfarin on factor VIIa in our study. A further study using a comparison group given aspirin alone is needed to determine the contribution of aspirin, if any, to the reduction in factor VIIa levels.

The effect of low-dose warfarin-aspirin on factor VIIa appeared to be waning over the 8 weeks of treatment (Fig 1), particularly in the last week (Figs 1 and 2). However, a statistically significant difference was not detected for the mean factor VIIa levels on weeks 3 through 8, and the 95% CI for these means overlap widely. The apparent waning could be caused by chance variation or a real biologic phenomenon, and should be investigated further.

There was a relatively wide range in the level of factor VIIa before treatment in our patients with stable coronary artery disease (range, 1.9 to 7.9 ng/mL). It is possible that...
factor VIIa may be potentially useful as a prognostic marker for myocardial infarction or other thromboembolic events in these patients. There was also a relatively wide range in the level of factor VIIa at INR values between 1.0 and 1.25 (Fig 3). This INR range represents the anticoagulant intensity achieved in 21% of patients treated with 3 mg of warfarin and in most patients treated with a fixed dose of 1 mg.\textsuperscript{18,19,22,46} The measurement of factor VIIa could be potentially useful for distinguishing patient groups at differing risk of thromboembolism during treatment with warfarin in doses which produce minimal changes in the INR. The assay for factor VIIa is easily performed using automated equipment currently available in most clinical coagulation laboratories, and does not require special blood collection or processing procedures.\textsuperscript{27} The potential clinical applications outlined above should be evaluated by prospective studies.

The results of our study support and extend the observations of Poller et al\textsuperscript{15,37} who reported factor VII activity was significantly higher 4 days after stopping oral anticoagulant treatment with nicoumalone than it was 6 weeks later. However, the factor VII activity before treatment in these patients was unknown. Our findings using a specific assay for factor VIIa support the possibility of a rebound to higher than pre-treatment levels. Further studies are necessary to clarify the time course of the potential rebound, and to determine if rebound occurs with factors II, IX, and X. The clinical importance of the potential rebound in factor VIIa is uncertain. Recently, Palareti et al reported that abrupt withdrawal of warfarin treatment is associated with ongoing activation of clotting as indicated by elevated levels of prothrombin fragment F\textsubscript{1+2} and thrombin-antithrombin III complexes.\textsuperscript{99} This ongoing clotting activation can be particularly intense in some patients.\textsuperscript{49} A hypothesis, to be tested in future studies, is that abrupt withdrawal of warfarin may be associated with a large rebound in factor VIIa or other vitamin K-dependent factors in some patients, leading to an intense activation of clotting and an increased risk of recurrent thromboembolism. It has been suggested that tapering the warfarin dose rather than abruptly stopping therapy will prevent the rebound phenomenon.\textsuperscript{28,29,48,89} However, the need to taper the dose is uncertain because it is unknown if this maneuver reduces the incidence of subsequent thromboembolic events. Further clinical trials are required to resolve these issues.

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