Plasma Levels of Protein C and Vitamin K-Dependent Coagulation Factors in Patients on Long-Term Oral Anticoagulant Therapy

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Plasma levels of protein C (PC) and vitamin K-dependent coagulation factors (factors II, VII, IX and X) were measured in 100 specimens from patients on long-term warfarin therapy. Both activities and antigens of these coagulation factors were decreased, depending on the thrombotest values. Factor II activity/antigen ratio and factor X activity/antigen ratio were correlated well with thrombotest values, indicating that the concentration of inactive molecules (PIVKAs) relative to normal proteins increases with increasing intensity of anticoagulation. Although PC antigen (PC: Ag) was also decreased, the ratios between PC: Ag and vitamin K-dependent coagulation factor antigens remained constant, being independent of the intensity of warfarin therapy. These findings indicate that long-term oral anticoagulant therapy results in the suppression of the synthesis of both vitamin K-dependent coagulation factors and PC, but the production of the coagulant and anticoagulant proteins is well-balanced.

Sodium warfarin, a vitamin K antagonist, has been widely used for the prophylactic treatment of the thromboembolic disorders. This oral anticoagulant inhibits the vitamin K-dependent protein synthesis in the liver, and modifies the plasma levels of vitamin K-dependent coagulation factors; factors II (prothrombin), VII, IX and X (Loelinger et al. 1963; Kazmier et al. 1965; Denson 1971). Protein C (PC) possesses potent anticoagulant properties through inactivation of...
factors Va and VIIIa (Kisiel et al. 1977; Marlar et al. 1982), and also enhances fibrinolytic activity by decreasing plasminogen activator inhibitor activity (van Hinsbergh et al. 1985; Sakata et al. 1985). The synthesis of PC is dependent on the presence of vitamin K (Stenflo 1976), and the levels of plasma PC will be depressed during warfarin therapy (Griffin et al. 1981; Bertina et al. 1982, 1984; Vigano et al. 1984). Thus, it is necessary to assess the behavior of PC and vitamin K-dependent coagulation factors simultaneously. In the present study, the plasma levels of these factors were systematically measured in patients on long-term warfarin therapy. PC antigen (PC : Ag), factor IX antigen (IX : Ag) and factor X antigen (X : Ag) were quantitated with a sensitive and accurate enzyme-linked immunosorbent assay (ELISA).

**MATERIALS AND METHODS**

**Collection of blood and preparation of plasma**

Venous blood was collected from patients with coronary heart disease, venous thromboembolism, or thrombophlebitis on long-term warfarin therapy. The patients were studied when the dosage of warfarin was stable for more than one month. Blood samples were anticoagulated with one-tenth volume of 0.11 M trisodium citrate for thrombotest determinations or one-tenth volume of 0.129 M trisodium citrate for other coagulation tests. Platelet-poor plasma was prepared from whole blood anticoagulated with trisodium citrate (12.9 mM, final concentration) by centrifugation at 2,000 ×g for 20 min at 4°C. Normal plasma pooled from 30 healthy subjects was used as reference plasma, which was defined to contain 100% activities or antigens of coagulation factors and PC.

**Hemostatic assay methods**

Thrombotest was performed on whole blood using a reagent from Nyegaard, Oslo, Norway.

Factors II, VII, IX and X activities (II : C, VII : C, IX : C and X : C) were measured by the standard one-stage assays (Biggs 1976). Factor II antigen (II : Ag) was quantitated by rocket immunoelectrophoresis (Laurell 1972) in 1% agarose gel (Sigma Chemical Co., St. Louis, MO, USA) prepared in 0.03 M barbital buffer, pH 8.6, containing 5 mM EDTA and 1% rabbit antisera (Behringwerke AG, Marburg, West Germany). Plasma samples (5 μl, diluted 1 : 5 in buffer) were applied in the wells and electrophoresis was performed at 1.2 mA/cm for 20 hr at 6°C. After electrophoresis, plates were washed in 0.15 M NaCl, dried, and stained with Coomassie brilliant blue.

Factor VII antigen (VII : Ag) was measured by an inhibitor neutralization assay using a rabbit antisera against human factor VII (Behringwerke AG) as described (Denson 1971; Biggs 1976).

IX : Ag, X : Ag and PC : Ag were quantitated by the ELISA methods (Asserachrom; Boehringer Mannheim Yamanouchi, Tokyo). The assays were performed similarly to the previously described procedure (Amiral et al. 1984; Takahashi et al. 1985).

**Statistical analysis**

Values were given in terms of means±s.d. Regression analysis was performed by the method of least squares, and the correlation coefficient (r) was calculated.
RESULTS

Vitamin K-dependent coagulation factors and PC were analyzed in 100 plasma specimens from patients on long-term warfarin therapy who showed thrombotest values equal to or lower than 50% activity. Thrombotest values ranged from 7 to 50%, with a mean ± S.D. of 23.5 ± 11.1%. The mean ± S.D. plasma level of II : C was 45.0 ± 19.7%; II : Ag, 61.1 ± 16.6%; VII : C, 45.9 ± 18.3%; VII : Ag, 63.8 ± 19.1%; IX : C, 68.2 ± 26.9%; IX : Ag, 81.6 ± 23.2%; X : C, 45.9 ± 25.4%; and X : Ag, 73.2 ± 20.5%. Thus factor IX was decreased less than factors II, VII and X. Each coagulation factor activity was correlated well with the corresponding antigen level. The correlation coefficient (r) between clotting activity and antigen was 0.837 (p < 0.01) in factor II, 0.614 (p < 0.01) in factor VII, 0.659 (p < 0.01) in factor IX, and 0.763 (p < 0.01) in factor X. The mean ± S.D. ratio of II : C to II : Ag was 0.72 ± 0.19; VII : C/VII : Ag, 0.74 ± 0.26; IX : C/IX : Ag, 0.84 ± 0.25; and X : C/X : Ag, 0.60 ± 0.23. The levels of activity and antigen of each coagulation factor were positively correlated with thrombotest values (Table 1); these values were decreased, depending on the intensity of anticoagulant therapy. In addition, the activity to antigen ratios were positively correlated with thrombotest values (r values ranging from 0.329 to 0.794, p < 0.01 for each). Among them, II : C/II : Ag and X : C/X : Ag ratios showed strong correlations with thrombotest values (Fig. 1).

PC : Ag was also decreased in these patients. The mean value ± S.D. of PC : Ag was 71.1 ± 23.3% (range, 31–135%). PC : Ag levels were positively correlated with thrombotest values and each coagulation factor (Table 1). Fig. 2 depicts the correlation between PC : Ag and thrombotest. PC : Ag was decreased, with

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation with TT</th>
<th>Correlation with PC : Ag</th>
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<tbody>
<tr>
<td>TT</td>
<td>—</td>
<td>0.565**</td>
</tr>
<tr>
<td>II : C</td>
<td>0.852**</td>
<td>0.587**</td>
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<tr>
<td>II : Ag</td>
<td>0.749**</td>
<td>0.670**</td>
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<tr>
<td>VII : C</td>
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<tr>
<td>VII : Ag</td>
<td>0.582**</td>
<td>0.500**</td>
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<tr>
<td>IX : C</td>
<td>0.576**</td>
<td>0.481**</td>
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<tr>
<td>IX : Ag</td>
<td>0.407**</td>
<td>0.482**</td>
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<tr>
<td>X : C</td>
<td>0.900**</td>
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<td>X : Ag</td>
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<td>0.589**</td>
</tr>
<tr>
<td>PC : Ag</td>
<td>0.565**</td>
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Correlation coefficients are given.

** p < 0.01.
increasing intensity of oral anticoagulant therapy. At the same time, however, a considerable variation in the levels of PC : Ag was observed among individuals with a similar intensity of anticoagulation. The relationship between PC : Ag and II : Ag was shown in Fig. 3. A relatively good correlation was obtained between these two parameters ($r = 0.670, p < 0.01$). The ratios between coagulation factor antigens and PC : Ag (II : Ag/PC : Ag, VII : Ag/PC : Ag, IX : Ag/PC : Ag and X : Ag/PC : Ag) were calculated. None of these four ratios were correlated with thrombotest values ($r = 0.096, 0.028, 0.191$ and 0.012, respectively).
These ratios at different intensity of warfarin therapy were shown in Table 2. Each ratio was nearly similar in all subgroups, being independent of the intensity of anticoagulation.

**DISCUSSION**

In the present study, the behavior of vitamin K-dependent proteins was systematically analyzed in patients on long-term oral anticoagulant therapy. Low thrombotest values were accompanied with low vitamin K-dependent coagulation factors, although the decrease in factor IX was relatively mild. The activity/antigen ratios of vitamin K-dependent coagulation factors were below 1.0, indicating the presence of circulating inactive molecules, PIVKAs (protein-induced by vitamin K antagonists or absence). The positive correlation of these
ratios to thrombotest values suggests that the concentration of PIVKAs relative to normal molecules increases with increasing intensity of the anticoagulation.

PC : Ag, a physiological anticoagulant, was decreased at the same time in warfarin-treated patients. It has been reported that, in patients starting oral anticoagulation, PC and factor VII decrease earlier than factors II, IX and X (Vigano et al. 1984), resulting in the transient paradoxical hypercoagulability. In patients on long-term therapy, the ratios between coagulant factor antigens and PC : Ag remained constant, independent of the intensity of the anticoagulation. Our results are in agreement with the findings of Bertina et al. (1982) who studied the II : Ag/PC : Ag and X : Ag/PC : Ag ratios in the similar patients. Our report demonstrated that in addition to these, VII : Ag/PC : Ag and IX : Ag/PC : Ag ratios remained constant, even when mildly anticoagulated patients were included for the analysis. Therefore, thrombotest and prothrombin time are still suitable measurements for monitoring the long-term oral anticoagulant therapy.

Warfarin treatment is thus accompanied by the decrease in PC to the levels similar to that observed in hereditary PC deficiency with thrombosis (Griffin et al. 1981; Bertina et al. 1982, 1984; Broekmans et al. 1983). However, clinical studies indicate that the net effect of vitamin K antagonists is antithrombotic due to the reduction of coagulation factors.

Acknowledgments

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References


