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DECLINE OF PROTEINS C AND S AND FACTORS II, VII, IX AND X DURING THE INITIATION OF WARFARIN THERAPY

P. Weiss, G.A. Soff, H. Halkin and U. Seligsohn.
Dept. of Medicine and Clinical Pharmacology unit, Sheba Medical Center, Tel-Hashomer; Institute of Hematology, Tel-Aviv Medical Center and Sackler School of Medicine, Tel-Aviv University, Israel.

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ABSTRACT

Oral anticoagulants achieve an antithrombotic effect only several days after initiation of treatment. A rapid decline of the vitamin-K dependent natural anticoagulants (proteins C and S) during this period might result in a prothrombic phase. We addressed this question by measuring the rates of decline of these proteins, as well as the vitamin K dependent procoagulants, in two groups of patients: A "high dose group" (n=7), who received a single 40 mg dose of warfarin, and a "low dose group" (n=20), who received daily individually adjusted doses. In the high dose group an early and marked decline of factor VII:C and protein C antigen was observed, while levels of the other vitamin K dependent factors were still relatively high. In the low dose group, all these proteins declined more gradually. Mean±SD of protein C antigen level at 46 hr was 56±12% in the low dose group, and only 44±6% ($p<0.05$) in the high dose group. We conclude that during the initiation of warfarin therapy there is a transient prothrombotic phase, which is less marked in patients given daily adjusted doses.

INTRODUCTION

The use of large loading doses of oral anticoagulants for the initiation of therapy was discontinued largely as a result of the work of O'Reilly and Aggeler in 1968 (1). These authors compared the rate of decline of the vitamin K dependent clotting factors in healthy subjects given either a single loading dose of 1.5 mg/kg body weight warfarin, or daily doses of 15 mg for a five day period. They found no significant differences between the two schedules, except for lower factor VII activity during the first 48 hr after the single large dose. As the role of factor VII in thrombogenesis was deemed questionable, the low daily doses were recommended

Key words: Warfarin, Clotting factors, Protein C, Protein S.

and accepted by most authorities (2,3).

Recently two new vitamin K dependent factors, proteins C and S, were discovered. Protein C, once activated, functions as a physiologic anticoagulant by proteolytically degrading activated factors V and VIII (4). Protein C is activated by thrombin in a complex with the endothelial cofactor thrombomodulin (5). Protein S acts probably as a cofactor of activated protein C by enhancing the binding of protein C to platelet surfaces (6). Accordingly, low levels of these proteins are associated with thrombosis (7-10).

The significance of the anticipated fall of protein C and S levels following initiation of warfarin therapy is as yet unknown. Conceivably, an early decline of protein C or S, preceding the fall of the vitamin K dependent procoagulants, might cause a prothrombotic phase. In this study we addressed these questions by comparing the rates of decline of the vitamin K dependent procoagulants and anticoagulants in patients who received either daily individually adjusted doses, or a single large dose of warfarin at the onset of therapy.

MATERIALS AND METHODS

Study groups:

1- High dose group. Seven consenting patients received a single loading dose of 40 mg warfarin at the initiation of treatment and no further doses for the next 72 hr.

2- Low dose group. Twenty patients received an initial dose of 10 mg warfarin and additional doses at 24 and 48 hr, according to their prothrombin time responses. Mean±SD dose at 24 hr was 7.7±3 mg and at 48 hr 5.5±3.3 mg. One patient received only the first 10 mg dose, as his prothrombin time ratio reached 2, and another patient did not require the last dose.

Indications for warfarin treatment were as follows: Prosthetic cardiac valve (14 patients), deep vein thrombosis or pulmonary embolism (7 patients) atrial fibrillation (4 patients) and unstable angina pectoris (2 patients). Seven patients were receiving heparin when warfarin was started.

Procedures:

Venous blood samples were drawn at 0 time and at 1,3,7,13,22,46 and 70 hr after the first warfarin dose. Nine parts of blood were mixed with one part of buffered citrate (consisting of 3 parts of 0.1M tri-sodium citrate and 2 parts of 0.1M citric acid). Plasma was separated within 2 hr of sampling and stored in small aliquots at -70°C until required for assay. Plastic ware was used throughout the study.

Laboratory methods:

All plasma samples were assayed for: Prothrombin time using rabbit brain thromboplastin (Dade), clotting activities of factors II, VII, IX and X, antigenicity of factors II, IX and X and antigenicity of protein C and S. Methods used were those previously described in our laboratory (9).

Protein S antigenicity was measured only in the high dose group by an electroimmunoassay, as described by Schwartz et al. (11), using an antibody kindly provided by Dr. J.H. Griffin (La Jolla, CA). All tests were done in duplicate. Prothrombin-time results were expressed as the prothrombin ratio (PTR), namely, the ratio between sample and pooled normal plasma prothrombin time. Other factors were expressed as percent activity or antigenicity of the individual patient level at time zero. Results were expressed as mean±SD throughout the text. Means were compared by unpaired t test where appropriate.

RESULTS

Changes in prothrombin time:

In both patient groups the increase in PTR was first evident after a lag period of 7 hr. In the high dose group PTR reached a peak value of 2.2±0.65 at 46 hr, with a subsequent decline to 1.7±0.27 at 70 hr. In the low dose group the rise in PTR was more gradual and persisted over the entire 70 hr period. The only significant difference between the two groups in PTR was at 46 hr (Fig. 1).

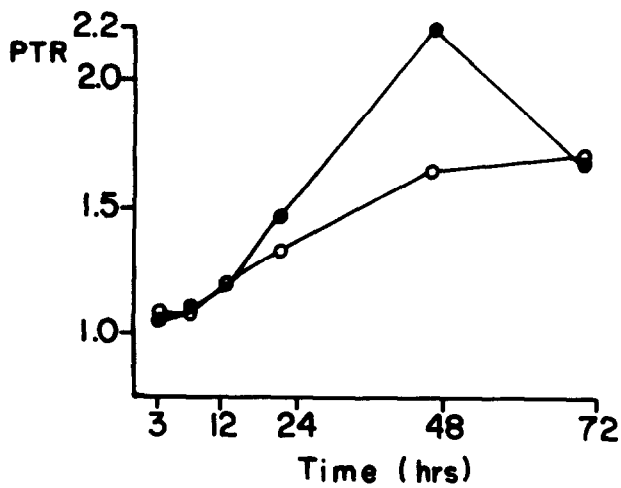

FIG. 1

Mean prothrombin time ratios (PTR) after warfarin loading with a single large dose -high dose group (•), or daily adjusted doses - low dose group (o)

Changes in individual procoagulants:

Levels of activity and antigenicity of factors II, VII (activity only), IX and X are depicted in Figures 2 and 3. All factor levels declined gradually except for factor VII in the high dose group, which reached a nadir of 5±4.4% at 46 hr and started to recover thereafter. There was an overall trend towards lower activity of all the vitamin K dependent factors in the high dose group, yet the differences were not significant, except for those in factor VII activity at 22 and 46 hr, and factor IX at

13 and 22 hr. In both groups antigenicity of the procoagulants declined more slowly than activity.

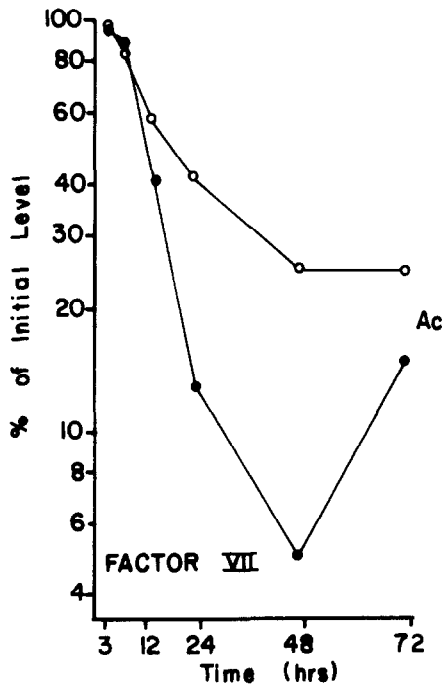

FIG. 2

Mean factor VII activity (Ac) after warfarin loading in the high dose group (•) and in the low dose group (o)

Changes in proteins C and S:

In the high dose group mean protein C antigen level reached a nadir at 46 hr, which differed significantly from that in the low dose group (44±6% vs. 56±12%, $p < 0.05$, Fig. 3). However, by 70 hr protein C levels were similar in both groups. Protein S antigen levels were measured only in the high dose group. They declined more gradually than protein C levels, also reaching a nadir at 46 hr (67±8.8%, Fig. 3).

Half-lives of the vitamin K dependent proteins:

In the high dose group individual semilogarithmic plots of clotting factor activity and protein C antigenicity versus time were linear between 3 to 13 or 22 hr. Decline half-lives were measured directly from these linear portions and the results are presented in Table 1. The mean±SD of protein-C half-life was 15.1±5.9 hr, intermediate to those of factors VII and IX. The decline of individual curves of protein S showed no linear segments, so that half-lives could not be calculated. In the low dose group individual curves also had no linear segments.

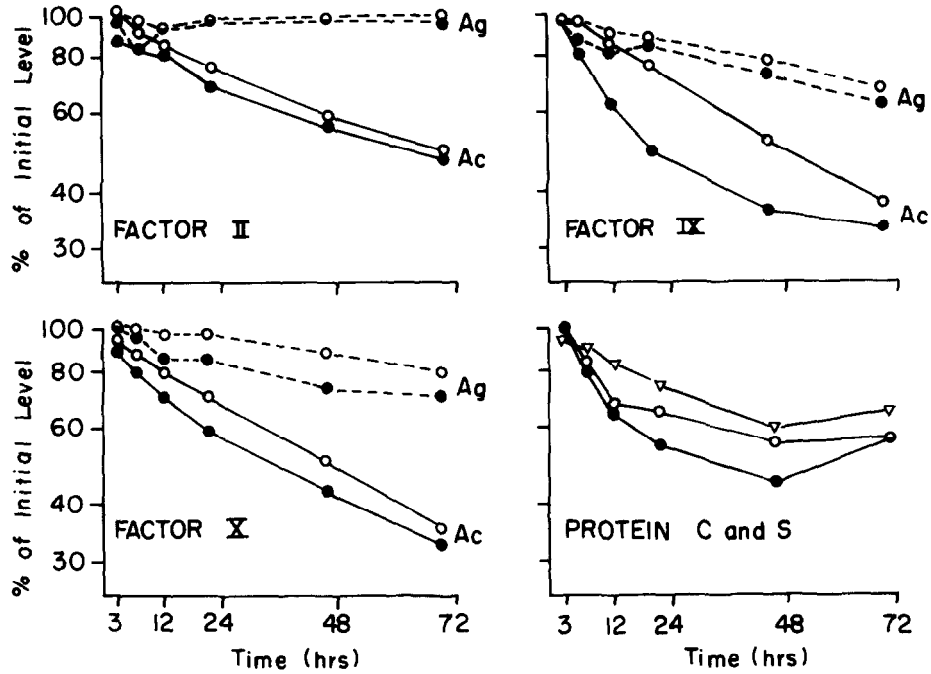

FIG. 3

Mean factor II, IX, X activity (Ac) and antigenicity (Ag) after warfarin loading in the high dose group (•) and in the low dose group (o). Mean protein C antigenicity for the two study groups and mean protein S antigenicity for the high dose group (▽) is also shown.

TABLE 1

Half-Lives (hr) of Factors II, VII, IX and X Activities and Protein C Antigenicity

Patient Number	Protein C Antigen	Activity of Factors II	VII	IX	X
1	11.5	55.5	5	9	32.5
2	12	37.5	7	54	34
3	16	61	4.5	-	15.5
4	12	32	4	21.5	32.5
5	26.5	51.5	5	17.5	30
6	18.5	29	5	10	22.5
7	9	28.5	5	15	20
Mean	15.1	42.1	5.1	21.2	26.7
S.D.	5.9	13.5	0.9	16.7	7.3

DISCUSSION

Following the initiation of warfarin treatment, levels of the vitamin K

dependent procoagulants (factors II, VII, IX and X) and anticoagulants (proteins C and S) decline at different rates, depending on the residual synthesis and degradation rate of each protein. A large enough loading dose can inhibit synthesis completely for a given time during which these elimination rates can be determined. Our study showed elimination half-lives of factors II, VII, IX and X similar to those reported in the literature (12). Our directly measured protein C antigen half-life (15.1±0.9 hr) was longer than earlier estimates (13, 14) but similar to that of a recent study (15). The lack of a linear segment for protein S decay probably indicates its addition to the plasma from a depot. Recently, it was shown that platelets as well as endothelial cells contain protein S (16, 17).

Another observation was that the antigenicity of the clotting factors declined more slowly than their activity - probably indicating ongoing synthesis of molecules lacking solely the final step of gamma-carboxylation as had previously been shown for factor II (18) and protein C (15).

Our results established once more that the first phase of anticoagulation after a large loading dose of warfarin is characterized mainly by factor VII deficiency (Fig. 2). Somewhat later significant deficiencies of proteins C and S were observed with mean nadir antigenicities of 44±6% and 67±8.8% respectively at 46 hr. The other vitamin K dependent factors declined more gradually. Initiation of warfarin therapy with the adjusted low doses also resulted in early falls in factor VII and protein C but to a lesser degree, and by 70 hr there were no significant differences between the effects of the two regimens.

The substantial factor VII deficiency and the increased PTR observed during the initiation of warfarin therapy do not provide protection against thrombosis (18). Conceivably, an antithrombotic effect can be achieved only after the other vitamin K procoagulants reach levels of about 25% (19). Our study and previous reports (20-22) showed that such antithrombotic levels cannot be achieved during the first days of treatment with either high or low doses of warfarin. Since patients with protein C levels of less than 65% of normals are at risk of thrombosis (8), the significant protein C antigen deficiency which we observed during the first days of warfarin treatment could be regarded as a prothrombotic phase which was further enhanced by the diminished protein S levels. Furthermore, Vigano D'Angelo et al. (15) recently demonstrated that under such circumstances the rate of decline of protein C anticoagulant activity substantially exceeds the decline rate of protein C antigen.

Since protein C decline was more marked in patients given large loading doses of warfarin, and as such doses did not achieve an antithrombotic state faster than small individually adjusted doses, the latter modality is preferable for the initiation of warfarin treatment. Our study further emphasizes that heparin administration is mandatory during the first days for an immediate antithrombotic effect as well as counteracting the possible effects of early falls in proteins C and S.

We are indebted to Dr. John H. Griffin, Scripps Research Foundation, La Jolla, for supplying us the antibody to protein S, and to Mrs. E. Zwang and Mrs. R. Eichel for their devoted and skillful technical assistance.

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