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Therapeutic Monitoring of Warfarin: the Appropriate Response Marker

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Abstract

Warfarin is a 4-hydroxycoumarin anticoagulant drug used for the prevention and management of thromboembolic and vascular diseases. It acts through the inhibition of the vitamin K-dependent transcarboxylation reactions that convert precursors of clotting factors into their active form. Appropriate use of warfarin requires patient monitoring and dosage adjustments, to ensure its safety and efficacy. The aim of this work was to clarify the relationship between traditional (prothrombin time, usually expressed as the international normalized ratio; INR) and alternative (clotting factors II and X) warfarin response markers to establish their usefulness for therapeutic drug monitoring.

Seventy adult outpatients, aged between 31 and 86 years old, were involved in the study. All subjects received warfarin in a monotherapy regimen and had been on a stable dosing schedule for at least two weeks to assure a steady-state condition. A total of 81 prothrombin times (expressed as INR), and factor II and factor X activity were simultaneously determined. Eleven patients presented repeated measurements at different time periods under the same dosing regimen. The results obtained from regression and cluster analysis showed a close relationship between factors II and X ($r = 0{\cdot}73$), a weak correlation between INR and both factor II ($r = -0{\cdot}35$) and factor X ($r = -0{\cdot}36$), and a very slight dependency between warfarin and the response markers used. In addition, it seems that independent of the selected response marker, in long-term warfarin therapy, reproducible responses can be obtained over time if a steady-state condition is achieved. The coefficients of variation for factors II and X were greater (35·44 and 37·93%, respectively) than INR (14·50%), indicating that INR is a more precise measure than either factor II or factor X.

In conclusion, INR appears to be the most appropriate warfarin response marker for therapeutic drug monitoring due to its universality, objectivity as a direct physiological effect measurement, and the available information regarding appropriate endpoints. However, when INR values are not in accordance with patient response therapy, factor II and factor X should be considered as an alternative to optimize warfarin therapy.

Warfarin is the most commonly used 4-hydroxycoumarin oral anticoagulant, although it presents large intra- and inter-individual variability with respect to kinetic and dynamic profiles, making a rational dosing regime difficult. A given dosing schedule may be completely inadequate in preventing thromboembolic events in one patient, but may cause serious haemorrhages in another patient (Hignite & Azarnoff 1980; Holford 1986; Murray et al 1987). The therapeutic effect of warfarin comes from its capacity to inhibit the action of vitamin K through the post-ribosomic blocking of vitamin K regeneration. Therefore, warfarin affects coagulation indirectly by impeding the effective recycling of vitamin K, essential for the activation of clotting factors II, VII, IX and X, but has no effect on their catabolism (Breckenridge 1977; Wessler & Gitel 1984).

Adequate monitoring of warfarin therapy is a challenge. The most commonly adopted marker for warfarin therapeutic monitoring in clinical practice is still prothrombin time, usually expressed as the international normalized ratio (INR) (Howard

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1994; Buck 1996). However, there is a time lag between warfarin administration and the beginning of the therapeutic response, which reflects the half-life of vitamin K-dependent clotting factors. During the first few days of warfarin therapy, prolongation of the prothrombin time primarily reflects the rapid decline in factor VII. This factor has the shortest half-life (4–6 h) of the vitamin K-dependent factors. During maintenance therapy the test is also sensitive to declining factor II and factor X levels (Hirsh et al 1992; Palareti & Legnani 1996).

Recently, increased attention has focussed on individual vitamin K-dependent clotting factors. It has been suggested that factor II and factor X determination may represent an alternative to monitoring warfarin therapy due to their long half-lives (approx. 3 and 2 days, respectively) and their role in the final step of the clotting cascade (common pathway). It also appears that factor II and factor X levels are the major determinants of the therapeutic efficacy of oral anticoagulants (Chan et al 1987; Porter & Sawyer 1992; Lind et al 1997).

In spite of the few clinical studies on clotting factors in long-term anticoagulant therapy, the available information suggests that factors II and X are closely related and can be a valuable guide for oral anticoagulant treatment (Lämmle et al 1980; Paul et al 1987; Hoppensteadt et al 1997; Lind et al 1997). The purpose of this study was to clarify the relationship between factor II, factor X and INR, and to analyse their usefulness as response markers for appropriate long-term warfarin therapeutic monitoring. The problems encountered in the management of anticoagulant therapy, including adverse effects and extended hospitalization for maintenance dose determination, demonstrate the need for a more effective approach to therapy.

Material and Methods

Subjects and blood sampling

Data was collected from 70 adult outpatients (33 male and 37 female), aged 63·60 ± 10·98 years (range 31–86 years), height 162·27 ± 9·64 cm (range 145–183 cm), and weight 70·60 ± 15·19 kg (range 37–120 kg), who had given previous consent to participate in the study. All patients received warfarin in a monotherapy regimen for not less than three months, and had been on a stable daily dose for at least two weeks before the sample used in the analysis was withdrawn, to assure a steady-state condition. The daily dose of warfarin ranged from 1·25 to 12·5 mg, and only patients with INR values inside the commonly adopted therapeutic range (2·0–3·5) were included in the data analysis (Howard 1994). Prothrombin times (expressed as INR), and factor II and factor X activity were simultaneously determined. Eleven patients showed values obtained at different times under the same dosing regimen, which allowed us to analyse the intra-individual behaviour.

Blood was collected (1 mL) and centrifuged (500 *g*), and the plasma was stored at −25°C. Before analysis, the plasma was rapidly defrosted by incubation at 37·4°C. Blood sampling times ranged from 1 to 25·5 h after dosing (median 13·5 h with an interquartile range of 12·5–16·0 h for a common once-a-day regimen), which was not relevant to the data analysis as little fluctuation was expected in the response markers during stable dosing under steady-state conditions (Wingard & Levy 1977).

Determination of prothrombin time, and factor II and factor X activity

Behring Fibrintimer II (calibrated according to the manufactures guidelines) and Behring coagulation reagents (Thromborel R, standard human plasma, factor II deficient plasma, factor X deficient plasma, and imidazol buffer) were used to perform the clotting assays of prothrombin time, and factor II and factor X activity. All samples were determined in duplicate.

In the prothrombin time screening test, 0·2 mL thromboplastin reagent (recombinant human thromboplastin and calcium chloride) was warmed to 37·4°C, and then forcibly added to 0·1 mL plasma. The time taken for clot formation (indicating fibrin formation) was recorded to the nearest tenth of a second. INR was directly calculated by the coagulation equipment, taking into account the prothrombin time of plasma sample, the control prothrombin time (experimentally measured value of standard human plasma reagent) and the specific sensitivity of the thromboplastin reagent (the International Sensitivity Index was 1·07).

Factors II and X were analysed using a one-stage clotting assay. The plasma sample was diluted 1 : 20 with imidazol buffer solution, and 0·1 mL factor II or factor X deficient plasma was added and incubated at 37·4°C for 1 min. Thromboplastin was then added and the clotting time was recorded. The factor deficient plasma supplied all the necessary factors except for factor II or factor X. The clotting time is dependent on the amount of the respective factor under measurement in the plasma sample. Factor II and factor X activity were expressed as the percentage activity relative to normal activity (70 to 120% according to Behring specifications). These values are determined indirectly from a

reference curve which plots coagulation time against a series of standard human plasma samples, prediluted with imidazol buffer (1 : 20; 1 : 40, 1 : 80, 1 : 160, which correspond, respectively, to 100, 50, 25 and 12·5% activity).

Statistical analysis

All statistical analyses were performed using Statistica software package. Linear regression analysis was carried out to assess the strength of the correlation between variables (factor II, factor X and INR). A cluster analysis was carried out to find a hierarchical tree involving response markers and warfarin input (daily dose); Euclidean distances were computed after a complete linkage (furthest neighbour). A paired *t*-test was used to compare the repeated measures of response markers observed in 11 patients, and $P < 0{\cdot}05$ was considered significant. Descriptive statistics and graphical analyses were performed when appropriate during the data analysis procedure.

Results

A total of 81 simultaneous determinations of factor II, factor X and INR were carried out in 70 patients (Table 1). Figures 1 and 2 show the weak relationship between INR and factors II and X ($r = -0{\cdot}35$ and $-0{\cdot}36$, respectively). The strongest correlation was found between factor II and factor X ($r = 0{\cdot}73$) (Figure 3) which confirms previous results (Lind et al 1997). The overall relationship between the warfarin response markers can be seen in Figure 4, where the surface plot (spline smoothing procedure) allows the interdependency between the variables to be analysed.

Figure 5 shows the tree plot obtained from a cluster analysis involving input (warfarin daily dose) and output (factor II, factor X and INR) covariates. The results are in agreement with the previous linear regression analysis, showing a close relationship between factor II and factor X, a weak correlation between both factors and INR, and a remote dependency between input (warfarin daily dose) and output (factor II, factor X and INR) covariates, which is in agreement with the literature (regardless of scope, i.e. pharmacokinetic and pharmacodynamic studies) (Mungall et al 1985; Holford 1986; Porter & Sawyer 1992; White et al 1995; Lind et al 1997).

From Figure 6, the scattering of each covariate can be analysed with the following associated

Table 1. Simultaneous determinations of factor II and factor X activity and INR in 70 patients.

Response marker	Mean ± s.d. (n = 81)	Range
Factor X (% activity)	23·62 ± 8·96	5·70–58·70
Factor II (% activity)	40·72 ± 14·43	16·10–88·80
INR (units)	2·62 ± 0·39	2·01–3·50

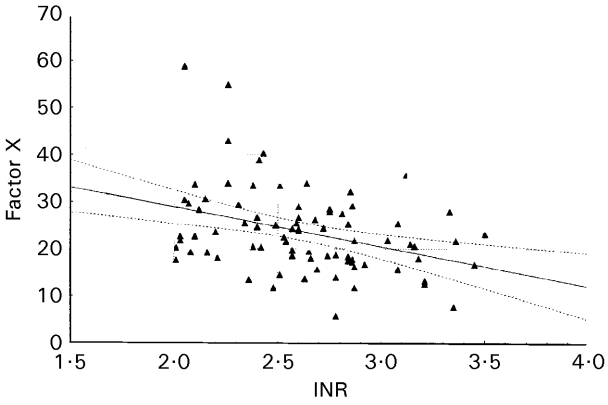

Figure 1. Relationship between international normalized ratio (INR) and factor X ($r = -0{\cdot}36$).

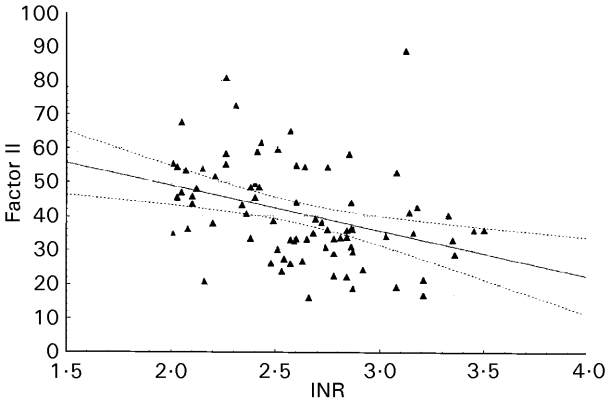

Figure 2. Relationship between international normalized ratio (INR) and factor II ($r = -0{\cdot}35$).

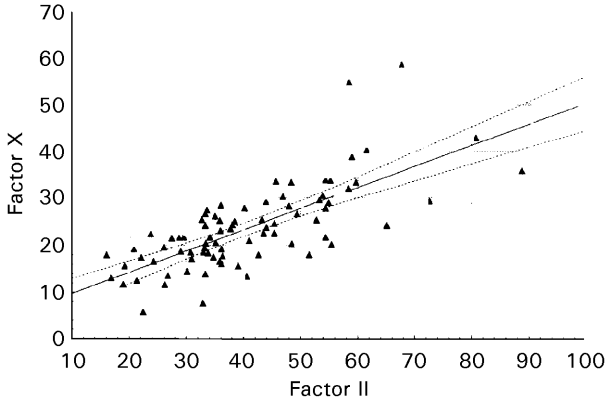

Figure 3. Relationship between factor II and factor X ($r = 0{\cdot}73$).

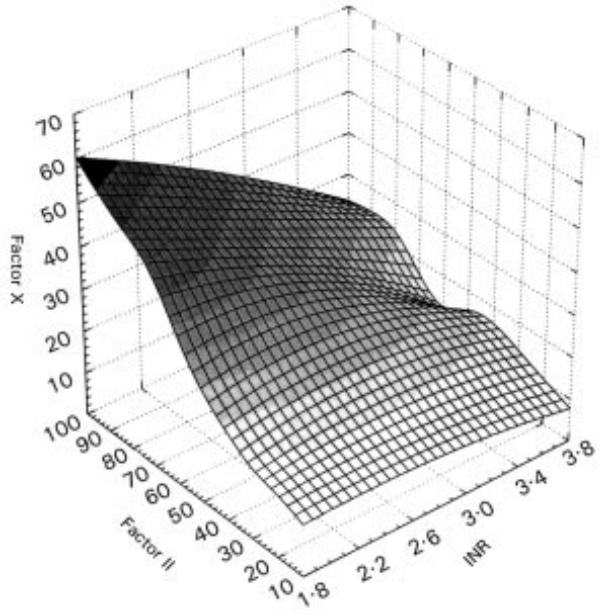

Figure 4. Surface plot (spline smoothing procedure) involving the available response markers, factor II, factor X and international normalized ratio (INR).

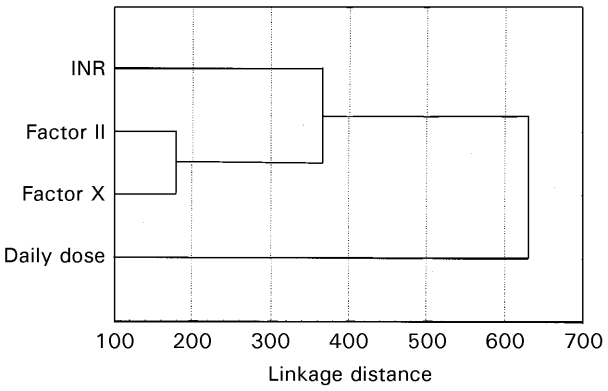

Figure 5. Horizontal hierarchical tree plot involving input (warfarin daily dose) and output (factor II, factor X and international normalized ratio; INR) covariates.

coefficients of variation: 47·74% (daily dose), 37·93% (factor X), 35·44% (factor II), and 14·50% (INR). From these results it seems that an acceptable effect (expressed as INR), in terms of accuracy and precision, can be obtained with individualized dosing schedules (warfarin daily dose). The coefficients of variation for factors II and X were more than twice the corresponding value for INR, indicating that INR (coagulation time) is perhaps a better measurement than other indirect measures (factor II and factor X) for warfarin monitoring therapy.

No significant differences were found when we compared repeated measures of response markers in 11 patients under the same dosing regimen at

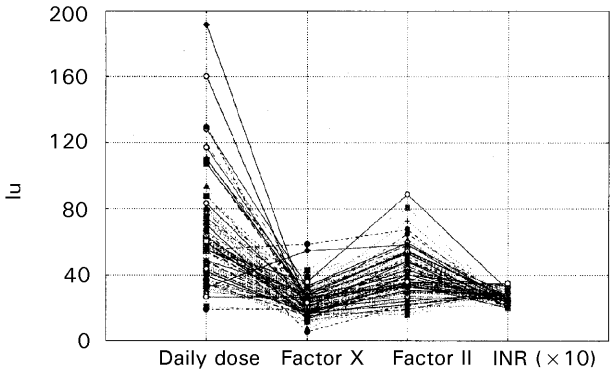

Figure 6. Graphical representation of input (warfarin daily dose) and output (factor X, factor II and international normalized ratio; INR) covariates.

Table 2. Comparison between the response markers in 11 patients at different times (repeated measurements).

Response marker	Time 1	Time 2
Factor X (% activity)	21·87 ± 13·49	23·46 ± 5·11
Factor II (% activity)	33·15 ± 14·46	37·66 ± 9·99
INR (units)	2·68 ± 0·39	2·55 ± 0·42

No significant differences were found between the results.

different times, which means that under the same steady-state conditions and after a posological readjustment/optimization, an appropriate response can be obtained over time (Table 2).

Discussion

The response to warfarin administration is highly variable and requires close monitoring to ensure its safety and efficacy (Wittkowsky 1997). This is based on its narrow therapeutic window, the serious consequences of dose-related toxicity or subtherapeutic response, the necessity to readjust therapeutic levels during treatment, and interindividual variability in the warfarin response (Theophanous & Barile 1973; Sawyer 1983; Iorio & Agnelli 1997).

Although the common marker of warfarin response is prothrombin time (usually expressed as INR), since oral anticoagulants are known to influence the functional concentrations of the clotting factors, it has been suggested that their direct measurement could be useful for predicting the extent of the anticoagulation and antithrombotic effect of these drugs in clinical practice (Hoppensteadt et al 1997).

In this work, we focussed on factors II and X because of their kinetic profile (long half-life), their

role in the final step of the clotting process and their influence on the warfarin antithrombotic effect (Chan et al 1987; Porter & Sawyer 1992; Lind et al 1997).

Slight differences between factor II and factor X median values were found (36·30 and 22·40% activity, respectively). However, the strong correlation between factors II and X ($r = 0{\cdot}73$) suggests a close relationship, potentially useful from a clinical point of view, which is in agreement with previously studies (Paul et al 1987; Lind et al 1997). Although the appropriate therapeutic range for each clotting factor has not yet been clearly established, it seems that values between 15 and 25% for factor X and between 20 and 40% for factor II, could be considered acceptable (Lämmle et al 1980; Aiach et al 1982).

In this study, the activity of factors II and X ranged from 16·10 to 88·80% and from 5·70 to 58·70%, respectively, and the INR values were inside the accepted therapeutic range (2·0–3·5). The practical consequences of this observation include the weak correlation shown by the regression analysis between INR and both factor II and factor X, indicating that we were unable to predict factor II or X from INR, and vice-versa. From a clinical point of view it means that INR is the best response marker to choose for several reasons: it is a direct physiological response marker; it is a universally accepted measurement; and it has a defined therapeutic window. However, factor II and factor X determination could be suitable alternatives when INR values are not consistent with the therapeutic effect.

Independent of the selected response marker, it seems that in long-term warfarin therapy (maintaining the same dosing schedule), the intra-individual variability is not a constraint given the results obtained at different times in 11 patients. For predictive purposes this is valuable because it means that under the same conditions, reproducible responses can be obtained over time. Otherwise, effort should be made to understand which demographic, clinical and pharmacological effects are involved in the inter-individual variability usually shown by warfarin treatment in clinical practice.

The absence of any significant linear relationship between input (warfarin daily dose) and output (factor II, factor X and INR) covariates was confirmed. There was very little correlation between warfarin daily dose and factor II ($r = 0{\cdot}15$), factor X ($r = -0{\cdot}03$), and INR ($r = -0{\cdot}01$), which explains the highly nonlinear pharmacokinetic/pharmacodynamic mechanistic models developed by other workers (Sawyer 1983; Holford 1986). Although the existing modelling work was carried out using prothrombin time as a warfarin response marker (or any other closely related marker, as happens with INR), our findings allow us to conclude that a nonlinear model will also be necessary to explain the relationship between warfarin administration and factor II and X covariates. This means that in terms of simplicity of correlation between input and output, factor II and factor X do not appear to represent any advantage over INR.

It appears that under steady-state conditions, INR seems to be the most appropriate response marker for warfarin therapeutic monitoring. However, factors II and X should be considered as an alternative when putative therapeutic INR values are not in accordance with the patient's response to therapy, as occurs when haemorrhagic or thromboembolic events are present.

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