Comparative Pharmacokinetics of Coumarin Anticoagulants XXVIII: Predictive Identification of Rats with Relatively Steep Serum Warfarin Concentration–Anticoagulant Response Characteristics

Keyphrases □ Warfarin—serum concentration related to anticoagulant response characteristics, rats □ Anticoagulants—serum warfarin concentration related to response characteristics, rats □ Prothrombin complex synthesis—related to serum warfarin concentration, rats

To the Editor:

The coumarin anticoagulants produce their effect by inhibiting the synthesis of the vitamin K-dependent clotting factors. There is an essentially linear relationship between the degree of inhibition of the prothrombin complex synthesis rate, R_{syn}, and the logarithm of the plasma or serum concentration of warfarin and dicumarol in humans (1, 2) and rats (3, 4). The slope of a plot of R_{syn} *versus* log drug concentration is negative and was designated by the letter m in previous publications. Studies in humans showed pronounced and quite reproducible interindividual differences in m (1, 2).

One may assume that individuals with high m values are particularly likely to be subject to excessive or inadequate anticoagulation, with the attendant risks, since a small change in the drug concentration in these subjects causes a large change in the anticoagulant effect. It is impractical and probably unwise to determine m values directly in patients at the start of anticoagulant therapy since this approach requires administration of a very large dose (about 10 times larger than the usual therapeutic dose). Since the rat also exhibits pronounced interindividual differences in m, we used this animal as a model to explore the possibility of prospectively identifying "steep" responders to warfarin (*i.e.*, those with a high m value).

Ten male Sprague–Dawley rats received a single 0.6-mg/kg iv injection of ^{3}H-(*S*)-(−)-warfarin. Blood samples were collected periodically, and the concentration of warfarin and the prothrombin complex activity were determined in serum. A larger blood sample was obtained at the end of the experiment for determination of the serum free fraction of (*S*)-warfarin by equilibrium dialysis against pH 7.4 buffer containing 1.5 μg of (*S*)-warfarin/ml.

Another 10 rats received daily 0.083–0.098-mg/kg injections of (*S*)-warfarin for 13 days, the dose being adjusted to maintain a prothrombin complex activity of 30–40% of normal. On the 15th day, these rats received a single 0.6-mg/kg dose of (*S*)-warfarin, and studies were carried out as described in the previous paragraph.

The analytical procedures, the method for the determination of the serum free fraction of warfarin, and the calculations required to determine R_{syn} and m were described previously (1, 3). Separate experiments with a large dose of unlabeled (*S*)-(−)-warfarin and ^{3}H-(*S*)-(−)-warfarin, in which serum warfarin concentrations were determined both spectrophotometrically and by scintillation counting after extraction and TLC, demonstrated that the specific activity of the drug in serum remained constant with time after injection.

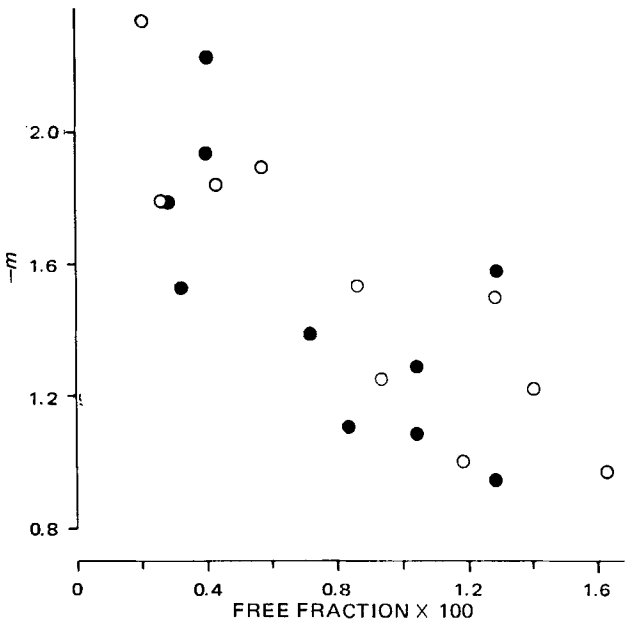

Figure 1—*Relationship between the slope of the relative anticoagulant effect–log warfarin concentration in the serum curve and the serum free fraction of* (S)-(−)-*warfarin in rats. Key:* ●, *acute dose; and* ○, *chronic dose.*

As shown in Fig. 1, m ranged from −0.939 to −2.33 and showed a statistically significant negative correlation with the serum free fraction value for warfarin ($r = -0.785$, $p < 0.001$). There was no apparent difference between acutely and chronically dosed animals. Thus, animals that showed the most extensive serum protein binding of warfarin also exhibited the steepest anticoagulant effect–log warfarin concentration curve. It is feasible, therefore, to identify predictively the steep responders to warfarin.

If the observations in rats can be confirmed in humans, it will be possible to identify, before institution of warfarin treatment, patients who have relatively steep anticoagulant effect–log serum warfarin concentration characteristics by taking a blood sample and determining the free fraction of warfarin in serum following *in vitro* addition of the drug. Previous studies showed more than fourfold differences in the serum free fraction of warfarin among 31 patients with cardiovascular disease who were being treated with warfarin (5).

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Estimation of Free Energy Change Associated with Conformational Transition of Proteins Showing Cooperative Binding Properties

Keyphrases □ Protein binding—log Q plot used to estimate free energy change associated with conformational transition □ Conformational transition—unbound to complexed proteins, log Q plot used to estimate free energy change □ Cooperative protein binding—log Q plot used to estimate free energy change associated with conformational transition □ Binding, cooperative protein—log Q plot used to estimate free energy change associated with conformational transition

To the Editor:

Sokoloski and Hoener (1) recently pointed out some corrections necessary in the use, as suggested by Ma *et al.* (2), of a log Q plot to determine protein–ligand affinity constants in systems where interaction between binding sites is indicated. These plots are usually used to calculate the relevant binding parameters following assumption of a model. However, in this article, we shall show that such plots can be used to evaluate a parameter that plays a key role in cooperative binding processes.

A protein–ligand system exhibiting positive interactions is used to illustrate the calculations. It is generally accepted, and supported by experimental evidence, that the protein must be able to assume at least two different conformations, depending upon the state of ligation (3, 4), for such cooperativity to occur. Let the conformation in the absence of ligand be the T conformation and that of the fully saturated protein be the R conformation. During ligation, the molecule changes its conformation from the T to the R form. Whether this process occurs stepwise or not is irrelevant to the current problem; Scheme I (where L is the ligand, n is the number of binding sites, and b is the experimentally observable ligation reaction) can be assumed to hold.

$$\begin{array}{ccc} T + nL & \overset{b}{\rightleftharpoons} & RL_n \\ {\scriptstyle a}\searrow & & \nearrow{\scriptstyle c} \\ & R + nL & \end{array}$$

Scheme I

The standard free energy change of this step can be considered to be composed of at least two other steps, namely the T to R conformational change as indicated by a and the binding of ligands to the R conformation as indicated by c in Scheme I. The standard free energy change, ΔF_a, of step a can be viewed as the driving force for cooperativity, its magnitude and variation with experimental conditions being important for understanding the molecular mechanism of cooperative effects. It is possible to estimate ΔF_a from a log Q plot as shown below.

The observed mean number of sites occupied is ν, so the fractional saturation, Y, is equal to ν/n, where n is the total number of binding sites. The free ligand concentration is x, allowing Q to be defined by (5):

$$\log Q = \log(Y/1 - Y) - \log x \qquad \text{(Eq. 1)}$$

where Q can be considered to be the apparent affinity constant at a given value of Y. In the case of a single site or of independent and equivalent sites, Q is a constant. Figure 1, constructed from the data of Tyuma *et al.* (6), illustrates the variation of log Q with Y when cooperativity occurs. If a model is assumed, then such a plot can be used to estimate successive binding constants. The method described in this article permits the evaluation of ΔF_a when suitable values for Y and x are available.

Wyman (7) showed that ΔF_b, the total standard free energy of ligand binding, is equal to $nRT \int_0^1 \ln x \, dY$. This true thermodynamic expression can be related to log Q as follows:

$$\int_0^1 \ln x \, dY = -\int_0^1 [\ln(Y/1 - Y) - \ln x] dY \qquad \text{(Eq. 2a)}$$

$$\int_0^1 \ln(Y/1 - Y) dY = 0 \qquad \text{(Eq. 2b)}$$

Therefore:

$$\Delta F_b = -nRT \int_0^1 \ln Q_b \, dY \qquad \text{(Eq. 3)}$$

where b indicates that Q is concerned with step b. A similar argument can be used to obtain:

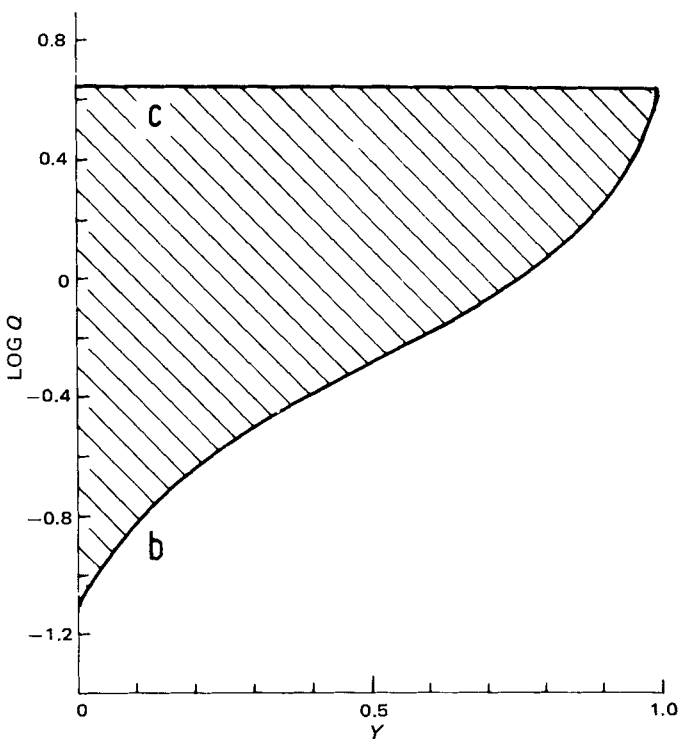

Figure 1—*Log Q plot of binding of oxygen to hemoglobin, calculated from the data in Ref. 6. In the calculation, partial oxygen pressure (in mm Hg) was used instead of molar oxygen concentration. Therefore, a conversion factor is involved in the calculation of* ΔF_b *or* ΔF_c *following the procedure outlined in the text. However, in the calculation of* ΔF_a, *this factor cancels out.*