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Effect of Plasma Protein Binding on Elimination of Warfarin

Keyphrases □ Warfarin—effect of plasma protein binding on elimination, pharmacokinetics □ Protein binding, plasma—effect on elimination of warfarin, pharmacokinetics □ Elimination, warfarin—effect of plasma protein binding, pharmacokinetics

To the Editor:

Studies in humans and rats have shown pronounced intersubject variation in the elimination of the coumarin anticoagulants dicumarol and warfarin (1–4). Detailed investigations in rats revealed that this intersubject variation may be associated with large differences in the distribution of these drugs in the body (3, 4). Similar changes in distribution and elimination were produced in perfused isolated rat liver preparations by varying the concentration of plasma proteins in the perfusion solution (5). These findings raised the possibility that some intersubject variation in the elimination of coumarin anticoagulant drugs may be related to differences in the binding of these very extensively protein bound drugs to plasma proteins. It is well recognized that differences in the activity of coumarin drug-metabolizing enzyme(s), particularly due to enzyme induction or inhibition, can also account for some intersubject variation in the elimination of these drugs (6, 7). The purposes of this communication are: *(a)* to present a theoretical pharmacokinetic formulation of the relationship between plasma protein binding and the kinetics of elimination of drugs such as dicumarol and warfarin, and *(b)* to describe, preliminary to a more detailed report, experimental data in support of the pharmacokinetic theory.

Let it be assumed that: *(a)* the kinetics of elimination are apparent first order, *(b)* elimination occurs from the central compartment, *(c)* the driving force of the rate-limiting steps of the elimination processes is the concentration of free (nonprotein bound) drug, *(d)* elimination is rate limited by these processes rather than by organ perfusion rate, *etc.*, and *(e)* the extent of plasma protein binding is essentially constant over the concentration range of therapeutic or experimental interest. The last assumption is realistic for drug concentrations at which only a small fraction of the binding sites on plasma proteins is occupied (8).

The sites of drug elimination (in the liver and elsewhere in the central compartment of the body) may be viewed as being surrounded by an aqueous solution of free drug. This aqueous solution represents a physiological space consisting of plasma water and other water exclusive of dissolved macromolecules capable of binding the drug. The volume of this space is designated as V_w. The rate of elimination, $-dA/dt$, of the drug is then proportional to the amount of drug[1] in V_w:

$$-dA/dt = k'fC_pV_w \qquad \text{(Eq. 1)}$$

where k' is a first-order elimination rate constant (which may be the sum of several rate constants for different elimination processes), which relates the amount of drug[1] in V_w to the rate of elimination[2]; C_p is the concentration of total (free and protein bound drug) in the plasma; and f is the fraction of free drug in the plasma. Designating the product of k' and V_w as k'' (which may be viewed as an intrinsic clearance constant) and converting $-dA/dt$ to a concentration term yield:

$$-(dC_p/dt)V_{area} = k''fC_p \qquad \text{(Eq. 2)}$$

where V_{area} is the volume of distribution of the drug in the body as previously defined (9). Unlike V_w, which is considered to be a physiological constant, V_{area} is a parameter that is affected by changes in protein binding. Rearrangement of Eq. 2 yields:

$$-(dC_p/dt)V_{area}C_p^{-1} = k''f \qquad \text{(Eq. 3)}$$

or:

$$\text{total plasma clearance} = k''f \qquad \text{(Eq. 4)}$$

Thus, a plot of the total plasma clearance of a drug *versus* the fraction of nonprotein bound drug in the plasma should be linear and go through the origin.

The elimination rate constant and the apparent volume of distribution of warfarin were determined in a selected group of 13 adult male Sprague–Dawley rats as previously described (4), except that the drug was administered intravenously. Serum protein binding[3] was determined by equilibrium dialysis using ^{14}C-warfarin. The fraction of free drug differed widely between animals (from 0.002 to 0.015), but it

[1] This is free drug only, by definition.
[2] The constant k' may also incorporate a partition coefficient to account for possible differences in the concentration of free drug in plasma water and other parts of V_w (such as intracellular water), perhaps due to differences in pH.
[3] Serum rather than plasma was used to prevent possible interference by anticoagulants in the protein binding determination.

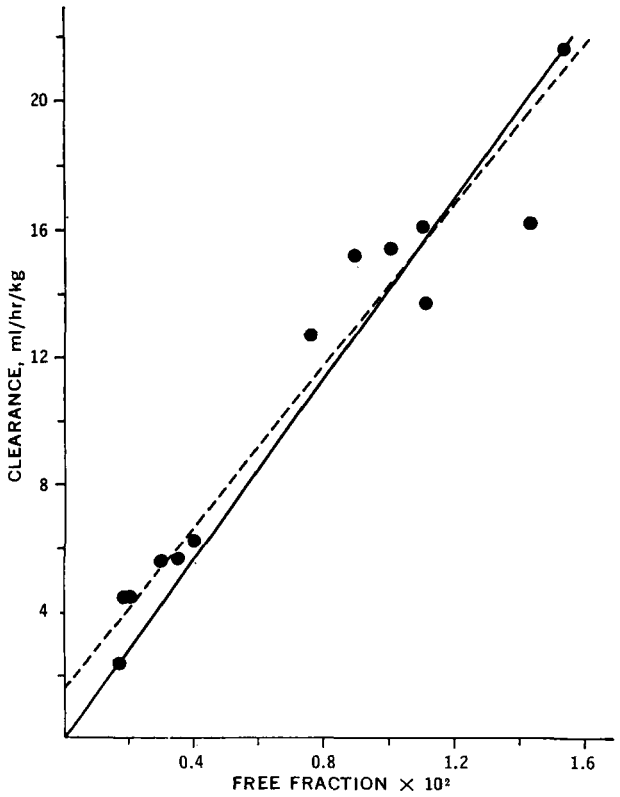

Figure 1—*Relationship between total plasma clearance of warfarin and fraction of free (not protein bound) drug in the serum of individual rats. These rats received intravenous doses of ^{14}C-warfarin, 0.6 mg/kg. Least-squares fit of the data yielded the stippled regression line; the continuous regression line was forced through the origin. The correlation coefficient is 0.97, $p < 0.001$.*

was essentially independent of total concentration for any one animal under the experimental conditions (total concentration, 0.3–4 μg/ml). Total plasma clearance[4] of warfarin ranged from about 2 to 22 ml hr^{-1} kg^{-1}. A plot of total clearance *versus* the fraction of free drug in the serum is linear, and the least-squares regression line intersects close to the origin, as predicted by Eq. 4 (Fig. 1).

In summary, our studies showed that individual differences in serum protein binding of warfarin (from 98.5 to 99.8%, equivalent to a range in the free fraction of from 0.002 to 0.015) can cause pronounced variations in the elimination rate constant (from 0.017 to 0.117 hr^{-1}) and apparent volume of distribution (from 137 to 206 ml/kg) of warfarin and that the effect of plasma protein binding on warfarin elimination can be quantified and predicted on the basis of a linear relationship between the total plasma clearance of warfarin and the free fraction of this drug in the serum. These observations may have clinical relevance in that *in vitro* protein binding studies with serum samples obtained from patients *prior to drug administration* may be useful for predicting the possibility of quantitatively unusual distribution, elimination, and pharmacological effect (4) characteristics of certain highly plasma protein bound drugs such as warfarin in individual patients.

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GLC Microdetermination of Plasma Anticonvulsant Levels

Keyphrases □ Anticonvulsants—plasma levels, GLC microdetermination □ GLC—microdetermination, anticonvulsant plasma levels

To the Editor:

The treatment of infants from birth for convulsive disorders is hindered by the lack of a rapid method for monitoring anticonvulsant blood levels using a small volume of plasma. The method of Berlin *et al.* (1) for diphenylhydantoin was modified by Solow[1] for the simultaneous determination of multiple drug levels. However, this method has a fairly low extraction efficiency for primidone, a primary anticonvulsant. Kållberg *et al.* (2) reported a rapid method for phenobarbital which we found also yielded almost total recovery of diphenylhydantoin. However, the recovery of primidone again was extremely low (16%), and the aqueous solution of trimethylphenylanilinium hydroxide, which was used for the final extraction,

[4] Total plasma clearance = $V_{area}\beta$, where the terminal slope of a plot of the logarithm of the drug concentration in plasma *versus* time equals $-\beta/2.3$.

[1] E. B. Solow, Indiana University, Indianapolis, IN 46202, personal communication.