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Comparative Pharmacokinetics of Coumarin Anticoagulants XXXI: Effect of Plasma Protein Binding on Distribution Kinetics of Dicumarol in Rats

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Abstract □ The purpose of this investigation was to determine, with respect to dicumarol, the effect of plasma protein binding on the pharmacokinetic parameters used conventionally to describe the distribution kinetics of a drug on the basis of the time course of its plasma concentration. After rapid intravenous injection, plasma dicumarol concentrations in adult male Sprague–Dawley rats declined triexponentially, with the terminal exponential phase starting at about 4 hr. The free fraction, f, of dicumarol in the serum of individual animals ranged from 0.000150 to 0.000790. The parameters of the equation $C_t = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ for plasma concentration C_t at time t were obtained by nonlinear least-squares computer fitting of the experimental data and varied appreciably between animals. Of these parameters, only β showed a significant correlation with f. These observations indicate that the distribution kinetics of this very extensively plasma protein-bound drug, as reflected by the time course of its plasma concentration after intravenous injection, are apparently not affected by intersubject differences in plasma protein binding. There is a remarkable similarity in the values of P, A, B, π, and α for dicumarol and warfarin, even though the serum free fraction of these drugs differs considerably.

Keyphrases □ Dicumarol—distribution kinetics, effect of plasma protein binding, rats □ Distribution kinetics—dicumarol, effect of plasma protein binding, rats □ Pharmacokinetics—distribution of dicumarol, effect of plasma protein binding, rats □ Protein binding, plasma—effect on distribution kinetics of dicumarol, rats □ Binding, plasma protein—effect on distribution kinetics of dicumarol, rats □ Anticoagulants—dicumarol, distribution kinetics, effect of plasma protein binding, rats

Serum (or plasma) protein binding can have a pronounced effect on the kinetics of drug elimination (1, 2). This effect is particularly striking with respect to the elimination of the extensively serum protein-bound anticoagulants warfarin and dicumarol by rats since the serum free fraction of these drugs varies over a wide range in these animals (3, 4). Consistent with theoretical considerations (5), the total clearance of warfarin and dicumarol is directly proportional to the serum free fraction of these drugs.

While the role of serum protein binding in drug elimination is beginning to be understood (1, 2), its effect on the kinetics of drug distribution from the blood to extravascular sites has not yet been elucidated. In a detailed study of the effect of serum protein binding on the distribution kinetics of warfarin in rats, no apparent relationship was found between the serum free fraction of warfarin and the pharmacokinetic parameters conventionally used to describe the distribution kinetics of a drug on the basis of a multiexponential decline of plasma drug concentrations after rapid intravenous injection (6). A similar study was initiated with dicumarol since its free fraction in rat serum is only about one-fiftieth that of warfarin (7). The results of this study and a comparison of the data obtained with dicumarol and warfarin are presented here.

EXPERIMENTAL

Single 3-ml blood samples were obtained from 40 adult male Sprague–Dawley rats, and the serum was separated. ^{14}C-Dicumarol was added to yield a concentration of 20 μg/ml, and the serum free fraction of dicumarol was determined by equilibrium dialysis (7). Ten animals with widely differing free fraction values were selected for further study. Two to 3 weeks later, a two-piece cannula of silicone rubber and polyethylene was implanted in their right jugular veins under light ether anesthesia (8, 9).

One or 2 days after cannulation, the rats were placed in individual metabolism cages with food and water freely available. A single dose of ^{14}C-dicumarol (specific activity of 61.7 μCi/mg), 8 mg/kg, was injected rapidly through the cannula. Blood samples (about 0.22 ml) were obtained at 5, 10, 20, 40, 60, and 120 min and then at less frequent intervals for a period equivalent to about three times the biological half-life of the drug in the particular animal. The blood samples were transferred immediately to heparinized micro blood collecting tubes[1], and the plasma was separated by centrifugation. The plasma dicumarol concentration was determined after selective extraction (10).

At the end of the experiment, a larger quantity of blood was obtained for the determination of the free fraction of dicumarol in serum. For this purpose, ^{14}C-dicumarol, 20 μg/ml, was added to the serum to yield a total concentration of 25–30 μg/ml and two or three serum samples from each rat were subjected to equilibrium dialysis (7).

The plasma dicumarol concentration data for individual animals were fitted to the triexponential equation $C_t = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ for plasma concentration C_t at time t by nonlinear least-squares regression (11). Convergence was defined as a relative change in the residual sum of squares $<10^{-4}$. Data in all functions were weighted numerically equal. Volume and clearance values were determined from the constants of the triexponential equation (12).

RESULTS

Plasma dicumarol concentrations declined exponentially with time after injection following an initial distribution phase. Data for the animals with the shortest and longest biological half-lives of dicumarol observed in this study are shown in Fig. 1. Data of similar quality were obtained from the other animals. A triexponential equation was required to describe the data; a biexponential equation was not adequate for this purpose.

The mean and range of the individual parameter values are listed in Table I. The intersubject distribution of the parameter values is shown in Fig. 2. The relatively widest range of values was observed with respect to A, π, and α; the least intersubject variation was found for B. However,

[1] Scientific Products, Evanston, Ill.

Table I—Dicumarol Kinetics in 10 Adult Male Sprague–Dawley Rats

Parameter	Mean	Range	*CV* of Individual Parameter Estimate[b]
P, μg/ml[a]	47.3	31.4–74.4	62.1 ± 59.3
A, μg/ml[a]	29.2	12.3–49.4	47.8 ± 46.0
B, μg/ml[a]	49.3	34.4–62.8	4.66 ± 1.86
π, hr^{-1}	10.0	3.38–18.8	80.8 ± 61.6
α, hr^{-1}	1.2	0.333–2.16	56.6 ± 44.8
β, hr^{-1}	0.0612	0.0269–0.0923	4.95 ± 1.66
V_c, ml/kg	65.2	45.2–83.7	
V_{area}, ml/kg	159	125–217	
$V_{intercept}$, ml/kg	166	128–233	
Total clearance, ml/hr kg	9.64	3.93–14.5	

[a] For an 8-mg/kg dose. [b] Mean ± *SD* of coefficient values for individual animals.

the estimated values for A, P, α, and π also had very large coefficients of variation (Table I). The drug has practically one-compartment characteristics if judged on the basis of the similarity of V_{area} and $V_{intercept}$.

The serum free fraction values for dicumarol ranged from 0.000150 to 0.000790. The relationship between serum free fraction and various pharmacokinetic constants is summarized in Table II. There is no apparent correlation between the serum free fraction of dicumarol and those constants that are thought to reflect (at least in part) the distribution kinetics of the drug: P, A, π, and α. As reported previously (4) and consistent with theoretical considerations (5), the free fraction values are strongly correlated with the total clearance of dicumarol. There is also a strong correlation between the free fraction and β.

Serum protein binding, as reflected by the serum free fraction values, had no apparent effect on the apparent volume of distribution of the hypothetical central compartment, V_c, for the drug (Table II and Fig. 3). This result is not an artifact of the data-fitting procedure since the earliest (5 min) plasma concentrations also showed no apparent relationship with the serum free fraction values (Fig. 3). This result is of interest since a strong, positive correlation between V_c and serum free fraction was found for warfarin in a previous study in which the protocol and methodology were almost identical to those of this investigation (6). Figure 4 shows the strong negative correlation between the warfarin concentrations 5 min after injection and the serum free fraction values of warfarin in 14 rats used in the previous study (6).

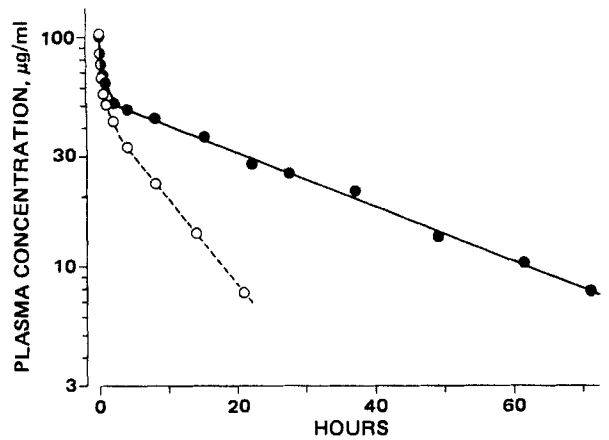

Figure 1—*Plasma dicumarol concentrations of two rats after 8 mg/kg iv of ^{14}C-dicumarol. The curves were fitted to the data by a nonlinear least-squares computer program.*

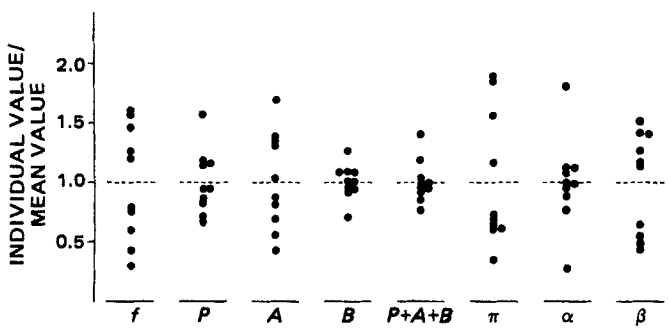

Figure 2—*Intersubject distribution of values for the parameters of the triexponential equation that describes the time course of plasma dicumarol concentrations and of values for the free fraction, f, of dicumarol in the serum of 10 rats.*

DISCUSSION

The results of this investigation show that serum protein binding, as reflected by the serum free fraction values, has no apparent effect on the pharmacokinetic parameters of dicumarol conventionally associated with the distribution kinetics of a drug: P, A, π, and α for the drug whose plasma concentrations decline triexponentially after intravenous injection. This conclusion must be viewed in the context that these parameters also have the greatest quantitative uncertainty, as reflected by the large coefficients of variation.

Dicumarol differs from warfarin in that serum protein binding has no apparent effect on the V_c of the former. The reason for this difference is not known. As discussed previously (6), there is no point in converting the directly determined pharmacokinetic constants of the triexponential equation to compartmental rate constants. Although this exercise was carried out as a matter of routine in the data analysis, the results provide no significant additional information and are not presented here.

The free fraction of warfarin is about 50 times larger than that of dicumarol when determined in the same serum samples (7). For both drugs, the free fraction value is essentially independent of concentration over a wide range (3, 4). It is informative, therefore, to compare the magnitude of the pharmacokinetic constants for the two drugs in rats. The mean

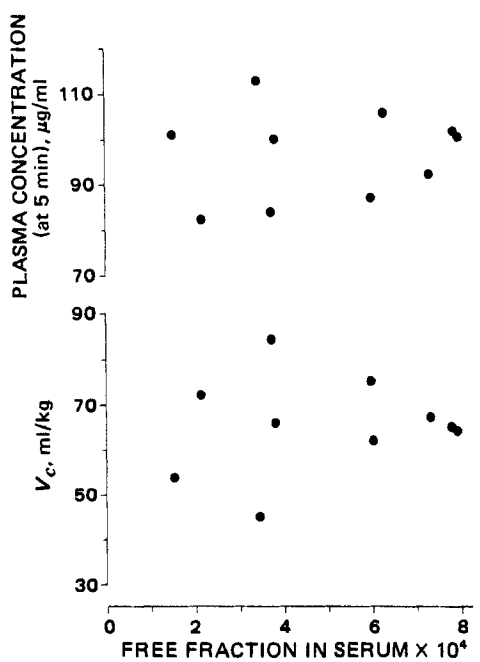

Figure 3—*Relationship between the serum free fraction of dicumarol and (a) plasma dicumarol concentration 5 min after intravenous injection (upper panel) and (b) the apparent volume of the hypothetical central compartment for dicumarol (lower panel) in 10 rats.*

Table II—Correlation between Pharmacokinetic Parameters for Dicumarol and the Serum Free Fraction of Dicumarol in 10 Rats

Parameter	r	Statistical Significance
P	0.187	N.S.
A	0.395	N.S.
B	0.162	N.S.
π	−0.615	N.S. ($p > 0.1$)
α	0.132	N.S.
β	0.892	$p < 0.001$
V_c	0.172	N.S.
V_{area}	0.242	N.S.
$V_{intercept}$	0.171	N.S.
Total clearance	0.831	$p < 0.005$

values of these constants are listed in Table III. Such a comparison cannot be rigorous since these data were obtained from different groups of animals (note, for example, that the free fraction ratio is about 25 rather than 50, which would be the expected ratio had the studies been performed in the same or in well-matched animals). Despite these limitations and the pronounced intersubject differences, the similarity in the mean values of all pharmacokinetic parameters[2] except β is striking. It may be that the distribution rate of both drugs from the blood to extravascular sites is limited by blood flow or diffusion, but this remains to be determined. Studies are now in progress with a drug much less extensively bound to serum proteins to determine if its distribution kinetics are different from those of dicumarol and warfarin.

Although dicumarol and warfarin have similar pharmacokinetic "distribution" parameter values (as shown in this study), almost identical total clearance values when determined in the same rats (7), and similar equieffective free plasma concentrations in terms of anticoagulant activity

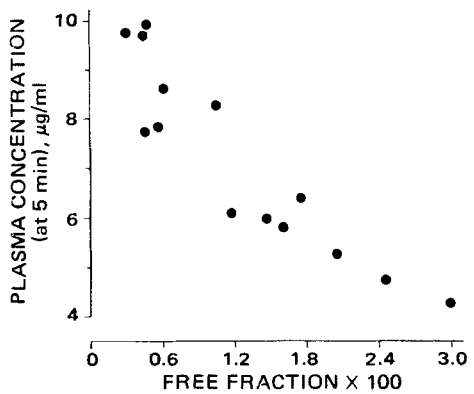

Figure 4—*Relationship between the serum free fraction of warfarin and plasma warfarin concentration of 14 rats 5 min after intravenous injection of warfarin, 0.51 mg/kg* (r = −0.913, p < 0.001).

[2] The serum free fraction value is not considered to be a "pharmacokinetic parameter" in the context of this discussion. The molecular weights of dicumarol and warfarin are similar so that P, A, and B need not be normalized on a molecular weight basis.

Table III—Comparison of Mean Values of Pharmacokinetic Parameters for Dicumarol and Warfarin in Rats

Parameter	Dicumarol[a]	Warfarin[b]
P, μg/ml[c]	5.91	9.16
A, μg/ml[c]	3.65	3.45
B, μg/ml[c]	6.16	6.04
π, hr^{-1}	10.0	6.98
α, hr^{-1}	1.20	1.03
β, hr^{-1}	0.0612	0.0310
Free fraction in serum × 10^4	4.97	123

[a] Results of this study, $n = 10$. [b] Results of a previous study (6), $n = 14$. [c] For a 1-mg/kg dose.

(7), these two drugs differ considerably in the extent of serum protein binding (7). Because of that difference and the similarity of their total clearance values, the intrinsic clearance (5) of dicumarol is much higher than that of warfarin. These comparisons are based on the "average" results of pharmacokinetic studies with racemic warfarin. While there are some quantitative differences in elimination kinetics and anticoagulant activity of (*R*)-(+)- and (*S*)-(−)-warfarin, respectively (13), these conclusions apply also when the intrinsic clearance of dicumarol is compared to that of the individual enantiomers of warfarin.

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