Comparative Pharmacokinetics of Coumarin Anticoagulants XXX: Relationship between Total Clearance and Serum Protein Binding of Dicumarol in Rats

CHII-MING LAI and GERHARD LEVY ˣ

Abstract □ The effect of serum protein binding on the elimination kinetics of dicumarol was studied. The serum free fraction of dicumarol was essentially independent of concentration over a wide concentration range and ranged from 0.00015 to 0.00079 in 10 adult rats. The total clearance of dicumarol in these animals ranged from 3.93 to 14.5 ml/kg/hr. As in previous studies, there was an excellent linear correlation between the elimination rate constant for dicumarol and the fraction of dicumarol in the liver (*i.e.*, the amount of drug in the liver divided by the amount of drug in the body). Consistent with theoretical considerations, there was a positive and apparently linear relationship between the total clearance and the serum free fraction of dicumarol. The individual serum free fraction and the fraction in liver values for dicumarol were strongly correlated. The pharmacokinetic model based on a proportional relationship between the apparent elimination rate constant and the fraction in the liver applies to dicumarol but not to warfarin and has limited utility. On the other hand, the model relating total clearance to the serum free fraction has been found to apply to dicumarol, warfarin, and other extensively plasma protein-bound drugs and can be utilized under clinical conditions.

Keyphrases □ Dicumarol—effect of serum protein binding on elimination kinetics, rats □ Protein binding, serum—dicumarol, effect on elimination kinetics, rats □ Binding, serum protein—dicumarol, effect on elimination kinetics, rats □ Elimination kinetics—dicumarol, effect of serum protein binding, rats □ Coumarin anticoagulants—dicumarol, effect of serum protein binding on elimination kinetics, rats □ Anticoagulants, coumarin—dicumarol, effect of serum protein binding on elimination kinetics, rats

There are pronounced intersubject differences in the elimination kinetics and in the serum protein binding of dicumarol in rats (1). The apparent first-order elimination rate constant for dicumarol in rats is proportional to the fraction of dicumarol in the liver, *i.e.*, to the amount of dicumarol in the liver divided by the amount of the drug in the entire body (2, 3). This proportionality is consistent with results of experiments with isolated perfused rat livers and with theoretical considerations that indicate that the biotransformation rate of dicumarol should be proportional to the amount of drug in the drug-metabolizing compartment, *i.e.*, the liver (4–6). However, subsequent studies with another coumarin anticoagulant, warfarin, failed to show any significant correlation between the elimination rate constant of that drug and its fraction in the liver (7).

Based on the results of the warfarin study, a theoretical model was developed which predicts a linear relationship between the total clearance and the serum free fraction of warfarin[1] (8). This model has been found to apply to warfarin in rats (7, 8) and humans (9), to bilirubin in rats (10), and to phenytoin in humans (11). Accordingly, this study was initiated to determine the relationship between the total clearance and the serum free fraction of dicumarol in rats.

[1] The serum free fraction is defined as the concentration of unbound drug divided by the concentration of total (free and bound) drug in serum.

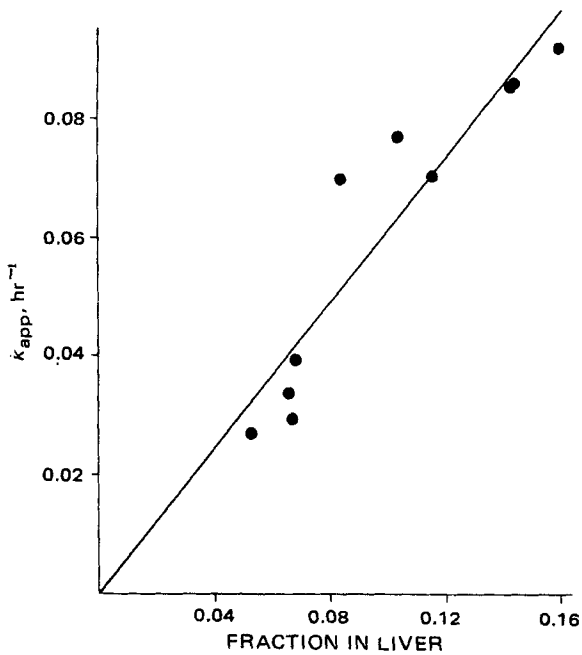

Figure 1—*Relationship between the apparent first-order elimination rate constant and the fraction of dicumarol in the liver (r = 0.931, p < 0.001).*

EXPERIMENTAL

Blood samples were obtained from 40 adult male Sprague–Dawley rats, and the serum was separated. ^{14}C-Dicumarol, 20 μg/ml, was added, and the serum free fraction was determined by equilibrium dialysis at 37° (1). Based on the results of this screening experiment, 10 rats with a wide and relatively even distribution of free fraction values were selected for further study. These animals, weighing 337–419 g, received a single 8-mg/kg iv dose of ^{14}C-dicumarol (specific activity, 61.7 μCi/mg).

Blood samples (0.22 ml) were obtained at 4–10-hr intervals (depending on the biological half-life) until the plasma dicumarol concentration had declined to about 8 μg/ml, at which time the animals were sacrificed by removing as much blood as possible from the aorta under ether anesthesia. The liver was then removed, blotted under slight pressure, and weighed. Dicumarol concentrations in plasma and liver were determined after selective extraction as previously described (12). The blood obtained when the animals were sacrificed was permitted to clot, and ^{14}C-dicumarol, 20 μg/ml, was added to the serum. The free fraction of dicumarol in these serum samples was determined by equilibrium dialysis (1). Pharmacokinetic analysis of the data was carried out as previously described (2).

Serum from five adult male Sprague–Dawley rats (who had not received dicumarol) was pooled to determine the relationship between the serum free fraction and the dicumarol concentration. ^{14}C-Dicumarol was added to yield serum samples with 10 different concentrations, ranging from about 9 to about 90 μg/ml. The serum free fraction of dicumarol in these samples was then determined in duplicate by equilibrium dialysis.

RESULTS

Results of the pharmacokinetic study are summarized in Table I. There were pronounced intersubject differences in the biological half-life, total

Table I—Pharmacokinetic Constants for Dicumarol in Adult Male Sprague–Dawley Rats

Rat	Half-Life, hr	Volume of Distribution, ml/kg	Total Clearance, ml/kg/hr	Free Fraction in Serum × 10^4	Fraction in Liver[a]	Liver/Serum Concentration Ratio[a]
1	25.8	149	4.00	1.50	0.0528	0.303
2	23.5	150	4.42	3.39	0.0670	0.341
3	20.6	179	6.02	2.13	0.0658	0.355
4	17.5	233	9.23	3.72	0.0679	0.481
5	9.80	128	9.05	6.25	0.115	0.427
6	9.90	162	11.3	3.79	0.0829	0.380
7	8.97	151	11.7	7.79	0.103	0.444
8	8.04	174	15.0	7.90	0.142	0.661
9	8.02	171	14.8	5.94	0.143	0.737
10	7.51	163	15.0	7.30	0.159	0.714

[a] At a plasma dicumarol concentration of about 8 μg/ml.

Table II—Relationship between Concentration and Free Fraction of Dicumarol in Pooled Serum from Five Untreated Rats[a]

Concentration, μg/ml	Free Fraction × 10^4
8.69	1.87
17.3	1.91
24.8	2.10
32.2	2.57
42.8	1.67
54.4	1.99
60.6	1.79
68.9	2.13
77.3	2.12
89.3	2.23

[a] Mean of two dialysis experiments for each concentration.

clearance, serum free fraction, and liver fraction of dicumarol and smaller differences in the apparent volume of distribution. Unlike warfarin (7), dicumarol had liver/serum concentration ratios less than unity under the experimental conditions. The free fraction of dicumarol in serum ranged from 0.00015 to 0.00079, indicating that the drug was 99.921–99.985% serum protein bound. The serum free fraction of dicumarol was independent of concentration over a wide concentration range (Table II). The coefficient of variation for the free fraction data summarized in Table II is 12.5%; the difference between duplicate samples was 14 ± 13.5% (mean ± *SD*), reflecting the technical difficulties of determining the serum protein binding of a very extensively bound drug.

As in previous studies (2, 3), there was a strong and apparently linear correlation between the apparent first-order elimination rate constant and the fraction of dicumarol in the liver (Fig. 1). However, this study also revealed a strong and apparent linear relationship between the total clearance and the free fraction of dicumarol in the serum (Fig. 2). There was a significant positive correlation between fraction in liver and serum free fraction values for dicumarol in individual animals ($r = 0.867$, $p < 0.003$, $n = 10$). No such correlation was found for warfarin (7).

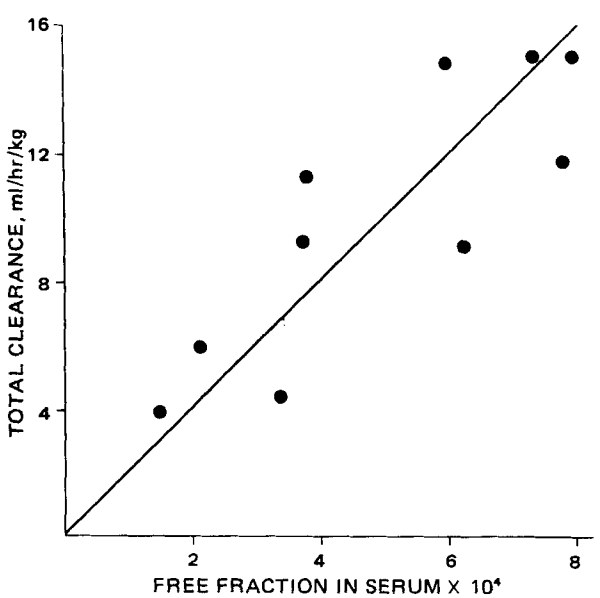

Figure 2—*Relationship between the total clearance and the serum free fraction of dicumarol* (r = 0.831, p < 0.005).

DISCUSSION

There is a strong theoretical basis and increasing experimental evidence for the concept that the total clearance of drugs subject to elimination by apparently linear biotransformation processes is proportional to their free fraction in serum unless clearance is so high as to be limited or affected by organ blood flow (13). Dicumarol is not subject to such limitation by blood flow through the liver (3, 14). The results of this study demonstrate that the total clearance of this very extensively serum protein-bound anticoagulant is proportional to its free fraction in serum and that serum protein binding is the major determinant of interindividual differences in the elimination of dicumarol by rats.

It is likely that such is the case also in humans, based on the following indirect evidence. There is a strong correlation between the total clearances of dicumarol and warfarin in individual rats (1). There is a similar strong correlation between individual serum free fraction values for dicumarol and warfarin in rats (1). Finally, the total clearance of warfarin in humans is proportional to the free fraction of the drug in serum (9). These observations, taken together, suggest that the total clearance of dicumarol in humans is likely to be proportional to its serum free fraction. Unlike the relationship between the apparent first-order elimination rate constant and the fraction of drug in the liver, the relationship between total clearance and the serum free fraction can be determined in humans under clinical conditions.

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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.