Comparative Pharmacokinetics of Coumarin Anticoagulants XV: Relationship between Pharmacokinetics of Dicumarol and Warfarin in Rats

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Abstract □ The distribution, elimination, and anticoagulant effect of dicumarol and warfarin were determined in adult male rats following intravenous injection of single doses of these drugs in crossover experiments. The biological half-life of dicumarol ranged from 5 to 28 hr; that of warfarin ranged from 9 to 30 hr. There was a statistically significant correlation between the following pharmacokinetic characteristics of dicumarol and warfarin in individual animals: biological half-life, apparent volume of distribution, total plasma clearance, and concentration in plasma eliciting one-half the maximum anticoagulant effect (effective concentration). The mean ratio of the respective biological half-lives (warfarin/dicumarol) was 1.42, and that of the apparent volumes of distribution was 1.50. The ratio of the effective plasma concentrations (dicumarol/warfarin) was correlated negatively with the half-life of dicumarol and positively with the ratio of the half-life values (warfarin/dicumarol) in individual animals. Additional studies with serum samples from other rats showed pronounced interindividual differences in the serum protein binding of both dicumarol and warfarin and a strong correlation between the protein binding of these two drugs in serum of individual animals. The results of this study, together with the results of previous studies in this series, indicate that serum protein binding is the major determinant of interindividual differences in the pharmacokinetics of dicumarol and warfarin in rats under these experimental conditions.

Keyphrases □ Dicumarol—distribution, half-life, elimination, anticoagulant effect determined, compared to warfarin, rats □ Warfarin—distribution, half-life, elimination, anticoagulant effect determined, compared to dicumarol, rats □ Coumarin anticoagulants—comparative pharmacokinetics of dicumarol and warfarin, rats □ Anticoagulants—comparative pharmacokinetics of dicumarol and warfarin, rats

The relationship between the elimination kinetics of different drugs in the same subjects has been the subject of a number of recent studies (1–4). The purposes of these studies were to explore the possible existence of common biotransformation pathways and genetic influences and to assess the feasibility of determining the elimination kinetics of one drug and using the results to predict the elimination kinetics of one or more other drugs in the same individual. The cited studies were limited generally to determinations of the biological half-life of the drugs. The study reported here was designed to obtain more comprehensive information about the relationship between the pharmacokinetic characteristics of two major anticoagulants, dicumarol and warfarin, in the same animals. Crossover studies were carried out in rats to determine the apparent volume of distribution, biological half-life, serum protein binding, and anticoagulant effect of these two drugs.

Table I—Biological Half-Life of Dicumarol (D) and Warfarin (W) in Individual Rats

Rat	Half-Life, hr		Ratio of Half-Lives (W/D)	Half-Life of Dicumarol in Screening Experiment, hr
	Dicumarol	Warfarin		
1[a]	5.12	9.72	1.90	4.75
2	6.54	8.89	1.36	5.96
3[a]	6.79	10.1	1.49	7.99
4[a]	11.6	16.7	1.44	11.3
5[a]	13.5	24.5	1.82	15.2
6	15.4	15.4	1.00	10.7
7	15.9	19.6	1.23	13.1
8	27.9	30.5	1.09	23.8
Mean			1.42	

[a] These animals received dicumarol first.

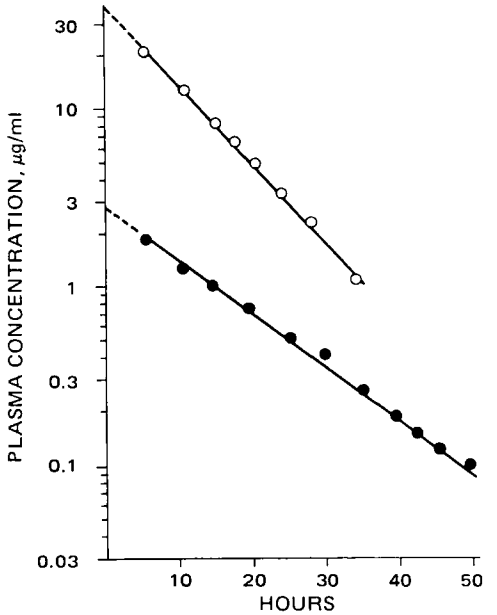

Figure 1—*Concentrations of dicumarol (○) and warfarin (●) in the plasma of a rat (Rat 3) as a function of time after administration of dicumarol, 6 mg/kg iv, and warfarin, 0.6 mg/kg iv, in separate experiments 3 weeks apart.*

EXPERIMENTAL

Thirty male Sprague–Dawley rats[1], 270–390 g, with unrestricted access to food[2] and water at all times, received ^{14}C-dicumarol, 6 mg/kg iv, in an initial screening experiment. The biological half-life of dicumarol was determined from the time course of drug concentrations in plasma (5). Eight animals, with dicumarol half-lives of 4.8–23.8 hr, were selected for further study 3 weeks later. The purpose of this selection process was to obtain a group of animals with a wide and relatively even distribution of dicumarol half-lives.

A crossover experiment was carried out in which four rats received dicumarol, 6 mg/kg iv, including about 30 μCi/kg of ^{14}C-dicumarol (10.9 μCi/mg). The other four rats received warfarin, 0.6 mg/kg iv, including about 26 μCi/kg of ^{14}C-warfarin (71 μCi/mg).

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Charles River formula 4RF.

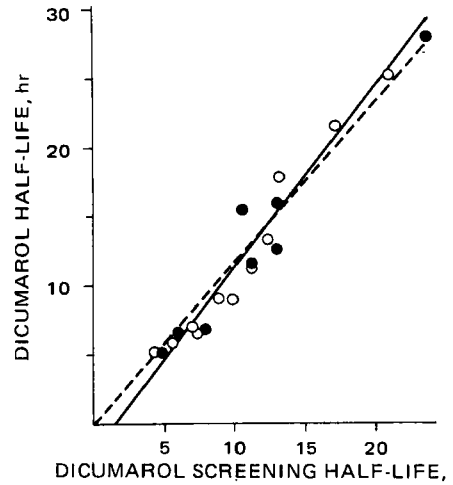

Figure 2—*Relationship between the biological half-life of dicumarol in the initial screening experiment and in the subsequent crossover study on the same animals (●). The open circles represent data for control animals from another study under the same conditions (to be published) (r = 0.967, p < 0.001). The continuous regression line was obtained by double regression analysis (11), and the stippled line was forced through the origin in this figure and in Figs. 3, 4, and 7.*

Three weeks later, the first group of animals received warfarin and the second received dicumarol. Serial blood samples (0.45 ml) were obtained after injection until the prothrombin complex activity returned to 65–100% of normal. Determinations of prothrombin complex activity, dicumarol and warfarin concentrations, and pharmacokinetic calculations were carried out as previously described (5–7).

Serum protein binding of dicumarol and warfarin was determined by equilibrium dialysis at 37° (8). Portions of serum from unmedicated individual rats were spiked with ^{14}C-warfarin (about 1 μg/ml) or ^{14}C-dicumarol[3] (about 20 μg/ml). A total of 21 rats was used. Warfarin concentrations were determined by scintillation counting after extraction and TLC (6, 7). Dicumarol concentrations were determined by scintillation counting after selective extraction (5) of samples from 10 rats and by scintillation counting after TLC of samples from the other 11 animals.

^{14}C-Dicumarol determinations by TLC were carried out by adding 0.1 ml of the serum or 1 ml of the dialysate sample to 0.1 ml of an aqueous solution of unlabeled dicumarol (400 μg/ml) in 0.15 *M* isotonic phosphate buffer, pH 7.4. This solution was acidified with 1 ml of 0.5 *N* hydrochloric acid and extracted into 2.5 ml of ethylene dichloride. After shaking for 45 min, the phases were separated by centrifugation at 1000×*g* for 3 min and the aqueous phase was removed by aspiration.

A 2-ml portion of the organic phase was placed in a vial[4] and evaporated at room temperature to dryness under a flow of nitrogen. The sides of the vial were washed down three times with chloroform, which was also evaporated. The residue was dissolved in 25 μl of chloroform and spotted on a silica gel TLC sheet[5] which had been activated (30 min at 100°) immediately before use. The vial was rinsed three times with 25-μl portions of chloroform, which were also applied to the TLC sheet. The sheet was developed immediately with toluene–ether–acetic acid (20:5:1) in a chamber which had been equilibrated with the solvent system. The development was stopped when the solvent front had advanced about 10 cm.

The dicumarol spot on the dried plate was visualized under UV light and identified as such by its R_f value (0.65). It was scraped off the TLC sheet and placed in a vial with 10 ml of scintillation fluid, and the radioactivity was determined[6] at 15° using an external standard. The recovery of dicumarol from serum samples con-

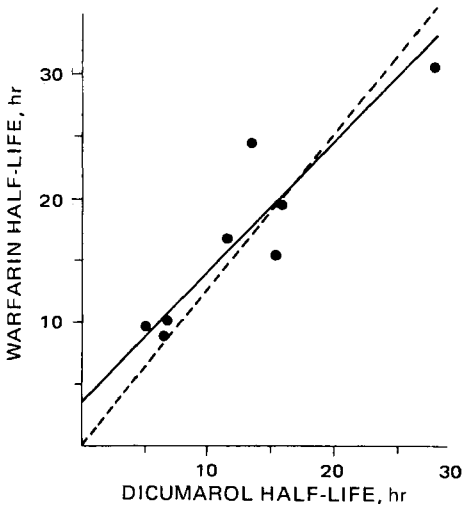

Figure 3—*Relationship between the biological half-life of dicumarol and warfarin in individual rats in the crossover study (r = 0.910, p < 0.005).*

Table II—Apparent Volume of Distribution and Total Plasma Clearance of Dicumarol (D) and Warfarin (W) in Individual Rats

Rat	Apparent Volume of Distribution, ml/kg			Total Plasma Clearance, ml/hr/kg		
	Dicumarol	Warfarin	Ratio (W/D)	Dicumarol	Warfarin	Ratio (W/D)
1	177	309	1.75	24.0	22.1	0.92
2	182	323	1.77	19.1	25.2	1.32
3	155	230	1.48	15.8	15.8	1.00
4	164	185	1.13	9.79	7.66	0.78
5	140	172	1.23	7.18	4.86	0.68
6	171	234	1.37	7.66	10.5	1.37
7	134	199	1.49	5.83	7.06	1.21
8	106	197	1.82	2.64	4.47	1.69
Mean			1.50			1.12

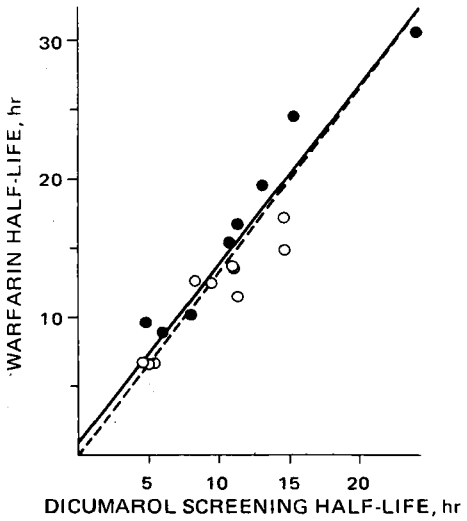

Figure 4—*Relationship between the biological half-life of dicumarol in the initial screening experiment and the biological half-life of warfarin in the subsequent crossover study on the same animals (●). The open circles represent data from another study (7) carried out under similar conditions except that the warfarin dose was 12 mg/kg (r = 0.936, p < 0.001).*

[3] Specific activity of 61.7 μCi/mg.

[4] Reacti-Vial, Pierce Chemical Co., Rockford, Ill.
[5] Silica gel F-254 TLC sheets, EM Laboratories, Elmsford, N.Y.
[6] Packard scintillation spectrometer, model 3320.

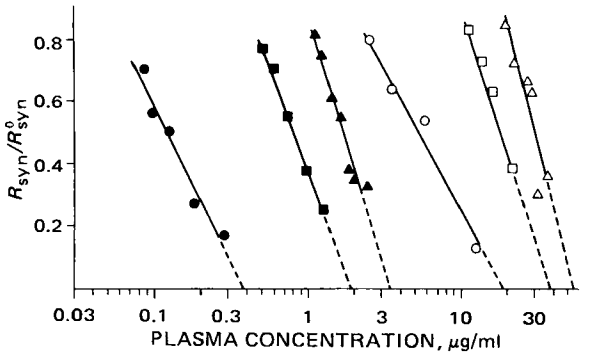

Figure 5—*Relationship between the anticoagulant effect (relative synthesis rate of prothrombin complex activity, expressed as R_{syn}/R^0_{syn}) and the concentrations of dicumarol (open symbols) and warfarin (solid symbols) in Rat 1 (circles), Rat 4 (squares), and Rat 8 (triangles).*

taining 0.01–48 μg/ml was independent of drug concentration and averaged 90.8 ± 2.4% (mean ± *SD*, n = 14). In preliminary experiments, excellent agreement was found between results when dicumarol-spiked serum samples were analyzed by both the selective extraction and TLC methods.

RESULTS

Figure 1 shows the time course of dicumarol and warfarin concentrations observed in one animal in separate experiments, 3 weeks apart. The biological half-life ($t_{1/2}$) of dicumarol in the individual rats ranged from 4.75 to 23.8 hr in the initial screening experiment and from 5.12 to 27.9 hr in the crossover study, while the $t_{1/2}$ of warfarin ranged from 8.89 to 30.5 hr (Table I). The apparent volume of distribution (V_d) of dicumarol in the crossover study ranged from 106 to 182 ml/kg, and the V_d of warfarin ranged from 172 to 323 ml/kg (Table II). There was a statistically significant positive correlation between the apparent first-order elimination rate constant (k_{el}) and V_d for dicumarol (r = 0.721, $p < 0.05$) and warfarin (r = 0.878, $p < 0.005$).

Figure 2 shows the relationship between the $t_{1/2}$ of dicumarol in the screening experiment and in the subsequent crossover study on the same animals. Included in the figure are 11 data points from control rats of another study. The correlation was very strong, and the average ratio of the individual $t_{1/2}$ values (crossover experiment/screening experiment) was 1.09. There was a strong and statistically highly significant correlation between the $t_{1/2}$ for dicumarol and warfarin in the crossover study (Fig. 3) and between the $t_{1/2}$ for dicumarol in the screening study and the $t_{1/2}$ for warfarin in the subsequent crossover study on the same animals (Fig. 4). The ratio of the warfarin $t_{1/2}$ to the dicumarol $t_{1/2}$ in the individual rats ranged from 1.00 to 1.90, with a mean of 1.42 (Table I).

The V_d of warfarin was 50% larger than that of dicumarol on the average (Table II), and the difference was highly statistically significant ($p < 0.005$ by paired t test). The correlation coefficient between the individual V_d values for dicumarol and warfarin was 0.696, but the correlation was not statistically significant in the eight animals. There was a strong correlation between the total plasma clearances of dicumarol and warfarin (r = 0.930, $p <$ 0.001); the mean ratio of the clearance values (warfarin/dicumarol) was 1.12 (Table II).

Figure 5 shows the relationship between the anticoagulant effect and the plasma concentrations of dicumarol and warfarin for three animals in this study. The regression lines were characterized by a slope (m) and by the plasma concentration at which the synthesis rate of prothrombin complex activity (R_{syn}) was one-half of its normal value (R^0_{syn}). That concentration is designated as $C_{50\%}$. The $-m$ and $C_{50\%}$ values for dicumarol and warfarin in all eight animals are listed in Table III.

There was a statistically significant correlation between $-m$ and $t_{1/2}$ for dicumarol (r = 0.920, $p < 0.001$) but not for warfarin (r = 0.470, $p > 0.2$). There also was a statistically significant correlation between $C_{50\%}$ and $t_{1/2}$ for dicumarol (r = 0.809, $p < 0.02$) and warfarin (r = 0.950, $p < 0.001$). Furthermore, there was a statistically significant negative correlation between $C_{50\%}$ and V_d with dicumarol (r = −0.789, $p < 0.02$) as well as warfarin (r = −0.707, $p <$ 0.05).

Table III—Relationship between Anticoagulant Effect (Inhibition of Prothrombin Complex Activity Synthesis Rate) and Plasma Concentration of Dicumarol (D) and Warfarin (W) in Individual Rats

Rat	—Slope[a] ($-m$), %/Day		Ratio of m Values (D/W)	Plasma Concentration when R_{syn}/R^0_{syn} = 0.5 μg/ml		Ratio of Concentrations (D/W)
	Dicumarol	Warfarin		Dicumarol	Warfarin	
1	0.932	1.02	0.91	5.41	0.123	44.0
2	0.899	1.11	0.81	5.62	0.177	31.8
3	0.854	1.52	0.56	8.19	0.217	37.7
4	1.63	1.38	1.18	18.8	0.813	23.1
5	1.46	1.42	1.03	28.5	0.830	34.3
6	1.41	1.69	0.83	14.0	0.599	23.4
7	1.66	1.13	1.47	13.9	0.725	19.2
8	2.06	1.63	1.26	30.5	1.63	17.6

[a] Slope of a plot of R_{syn}/R^0_{syn} *versus* log plasma concentration (m).

The average ratio of the individual m values (dicumarol/warfarin) was 1.01 ± 0.29, and there was no apparent correlation with the $t_{1/2}$ of either drug. This finding permits a determination of the relative potency of the two drugs on the basis of the ratio of their equieffective plasma concentrations ($C_{50\%}$). The ratio of the $C_{50\%}$ values (dicumarol/warfarin) ranged from 17.6 to 44.0 (Table III) and varied systematically with the $t_{1/2}$ of dicumarol (Fig. 6). This ratio also correlated with the ratio (warfarin/dicumarol) of the $t_{1/2}$ for the two drugs (r = 0.828, $p < 0.01$). There was a strong positive correlation between the $C_{50\%}$ values of dicumarol and warfarin in individual rats (r = 0.911, $p < 0.005$).

For technical reasons, serum protein binding of the two anticoagulants was determined in another group of 21 animals. These animals did not receive either dicumarol or warfarin; the drugs were added directly to the individual serum samples. There were pronounced interindividual differences in the serum protein binding of dicumarol and warfarin, but there was a strong correlation (r = 0.978, $p < 0.001$) between the free fraction (100 × free fraction = percent free) of the two drugs in the serum of the individual animals (Fig. 7). At the concentrations used, the ratio of free fraction values (warfarin/dicumarol) was 53.3 ± 7.0 (mean ± *SD*).

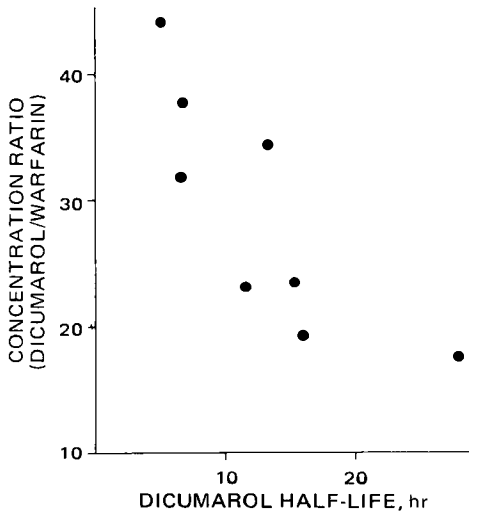

Figure 6—*Relationship between anticoagulant potency ratio (warfarin/dicumarol) [expressed as the ratio (dicumarol/warfarin) of plasma concentrations at which the rate of synthesis of prothrombin complex activity is 50% of normal] and biological half-life of dicumarol in individual rats (r = −0.800, $p < 0.02$).*

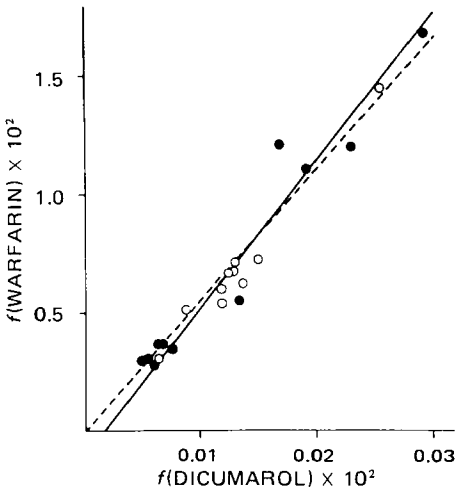

Figure 7—*Relationship between protein binding of dicumarol and warfarin in the serum of individual rats. Plotted are the free fractions (f) determined by equilibrium dialysis at 37°. Key: ●, data from 11 animals with dicumarol assayed by TLC; and ○, data from 10 other animals with dicumarol assayed by selective extraction method (5) (r = 0.978, p < 0.001). Dicumarol was 99.97–99.995% bound and warfarin was 98.3–99.7% bound to serum protein.*

DISCUSSION

The results of this investigation reveal a very strong correlation between dicumarol and warfarin with respect to their $t_{1/2}$, V_d, total plasma clearance, and serum protein binding characteristics in individual rats. The $t_{1/2}$ of warfarin was 42% longer than that of dicumarol, and the V_d of warfarin was 50% larger than that of dicumarol on the average. Consequently, the total plasma clearance of the two drugs was almost identical (Table II). The excellent reproducibility of the dicumarol $t_{1/2}$ values found in this investigation also was observed in a previous study (9); the $t_{1/2}$ of warfarin also showed excellent reproducibility in individual animals (7).

We have found that the interindividual differences in the elimination kinetics of warfarin are related to (and presumably caused by) differences in serum protein binding of the drug (8, 10). The higher the free fraction of warfarin, the shorter is the $t_{1/2}$ and the larger is the V_d of the drug (8). The results of this study suggest that the same relationship holds true for dicumarol (although the evidence is only indirect) and that the correlation of pharmacokinetic characteristics of the two drugs in individual animals is a consequence of the strong correlation of their serum protein binding characteristics.

Because of the relative constancy of the individual $t_{1/2}$ and V_d ratios, it is possible to compare the two drugs with respect to $t_{1/2}$ and V_d, despite pronounced interindividual variations in the value of these constants. It is much more difficult to compare the relative potency of dicumarol and warfarin. The ratio of equieffective plasma concentrations (dicumarol/warfarin) is higher in rapid metabolizers than in slow metabolizers of these drugs (Table III). The reasons for this systematic variation are complex and relate in part to the nonlinear serum protein binding characteristics of dicumarol[7], the different potency of the two enantiomers of warfarin (7), and the time-dependent change in the enantiomer concentration ratio following administration of racemic warfarin (7).

It is interesting, however, that in this group of rats warfarin was *approximately* 30 times as potent as dicumarol on the basis of the mean ratio of their equieffective concentrations[8], while the non-protein-bound fraction of warfarin was *approximately* 50 times larger than that of dicumarol. Thus, the two anticoagulants were almost equipotent in the rat if compared on the basis of their free (non-protein-bound) concentrations.

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[x] To whom inquiries should be directed.

[7] To be published.

[8] This refers to the concentrations at which R_{syn} is one-half of normal (*i.e.*, $C_{50\%}$). Moreover, the equieffective concentration ratio of dicumarol and warfarin differs in rapid and slow metabolizers of these drugs, and it is generally advisable to focus on individual values rather than on the average value of this ratio.