Comparative Pharmacokinetics of Coumarin Anticoagulants XXXII: Interindividual Differences in Binding of Warfarin and Dicumarol in Rat Liver and Implications for Physiological Pharmacokinetic Modeling

GERHARD LEVY ˣ, CHII-MING LAI, and AVRAHAM YACOBI

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Abstract □ This investigation was designed to determine the *in vivo* binding of racemic warfarin and dicumarol in the liver of individual adult male rats. The animals received single injections of one or the other drug. They were sacrificed after a period of time equivalent to several times the biological half-life of the drug, at plasma concentrations of 0.40 ± 0.10 (warfarin) or 7.7 ± 1.1 (dicumarol) μg/ml. Drug concentrations in the liver, serum, and serum water (*i.e.*, unbound drug in serum) were determined, and the concentration of unbound drug in the liver was calculated on the basis of the assumption that the concentrations of unbound drug in the serum and in liver water are equal. The free fraction of warfarin in the liver of 13 rats ranged from 0.06×10^{-2} to 0.6×10^{-2} and was substantially smaller than the warfarin free fraction in the serum. The free fraction of dicumarol in the liver of 10 rats ranged from 3.5×10^{-4} to 14×10^{-4} and was larger than the dicumarol free fraction in the serum. However, there was a statistically significant positive correlation between the serum and liver free fraction values of both drugs. Physiologically based pharmacokinetic modeling of protein-bound drugs, which requires estimation of protein-binding parameters in serum and tissues, must take account of the possibility of pronounced intersubject differences in the binding of such drugs in serum as well as tissues. With warfarin and dicumarol, tissue to plasma distribution ratios for liver and kidneys are much less variable (and, therefore, more suitable for pharmacokinetic modeling) than are the ratios of unbound to total concentration.

Keyphrases □ Warfarin—*in vivo* binding in rat liver, implications for pharmacokinetic modeling □ Dicumarol—*in vivo* binding in rat liver, implications for pharmacokinetic modeling □ Pharmacokinetics—warfarin and dicumarol, *in vivo* binding in rat liver, implications for modeling □ Coumarin anticoagulants—warfarin and dicumarol, *in vivo* binding in rat liver, implications for pharmacokinetic modeling □ Anticoagulants—warfarin and dicumarol, *in vivo* binding in rat liver, implications for pharmacokinetic modeling

The distribution of a drug in the body is affected by its binding to proteins in plasma as well as by its binding to tissues. Physiologically based pharmacokinetic models for drugs that are subject to such binding must incorporate parameters that relate the concentrations of free and bound drug in the various compartments (1, 2). While there is a large body of knowledge about the binding of drugs in plasma or serum, little is known about the binding of drugs in tissues (2).

One reason for this lack of knowledge is the virtual impossibility of performing meaningful tissue binding studies *in vitro*. It is unrealistic to equate the drug binding characteristics of tissue proteins with those of plasma albumin, and it is incorrect to assume that the binding parameters obtained with dilute protein solutions apply to high "concentrations" of proteins found in tissues (2). Even in the qualitative sense, *in vitro* studies of drug binding to tissues can be misleading. For example, phenylbutazone displaces dicumarol from plasma proteins but has no apparent effect on the *in vivo* liver to plasma concentration ratio of dicumarol in rats (3). This result indicates that phenylbutazone displaces dicumarol equally from plasma and tissue binding sites, yet *in vitro* studies with liver homogenate did not reveal any displacing effect of phenylbutazone on dicumarol (3).

Warfarin and dicumarol are very extensively protein bound in plasma or serum (4–6). They must also be extensively bound in tissues such as the liver, since their liver to serum concentration ratios can exceed unity (3, 7). Since these anticoagulants are eliminated almost entirely by biotransformation in the liver, the binding of the drugs in the liver is of particular interest. There are pronounced interindividual differences in the serum protein binding of warfarin and dicumarol in rats (4) and of warfarin in humans (5, 8). The interindividual variation of dicumarol serum protein binding has not been studied in humans but may be expected to be of similar magnitude as that of warfarin.

In view of this variability, there arises the question of whether the binding of these drugs in tissues is similarly variable. This question was addressed in the investigation described here. For physiologically based pharmacokinetic modeling, one may elect to use tissue to serum distribution ratios (9, 10). Therefore, the variability of these ratios for the two anticoagulants with respect to the liver and kidneys was also assessed.

EXPERIMENTAL

The experimental data reported here were obtained in conjunction with other investigations, and the protocols and experimental procedures already were described (7, 11, 12). Briefly, adult male Sprague–Dawley rats were selected by suitable screening tests to obtain groups of animals with a relatively wide distribution of warfarin or dicumarol free fraction values in serum. They received a single injection of warfarin, 0.6 mg/kg iv, or dicumarol, 6 or 8 mg/kg iv.

Blood samples were obtained periodically, and drug concentrations in plasma were determined promptly by specific and sensitive methods. These data were used to predict the time when warfarin concentrations had decreased by about 90% and dicumarol concentrations had decreased by about 80%. At the indicated time, the animals were exsanguinated from the aorta and the liver and kidneys were removed. These organs were blotted and pressed lightly to remove most of the remaining blood.

Drug concentrations were determined in the tissues after homogenization and extraction and in serum. The free fraction of warfarin or dicumarol in serum was determined by equilibrium dialysis. Duplicate serum free fraction determinations differed, on the average, by 6.4% for warfarin (13) and 14% for dicumarol (11).

The liver of an adult rat contains about 70% water (14). It was assumed that the concentration of free (unbound) drug in this tissue water is equal to the concentration of free drug in plasma or serum water. Therefore, the free fraction of warfarin or dicumarol in the liver may be estimated by dividing (0.7 × serum free fraction × total concentration in serum) by the total concentration of the drug in the liver. The free fraction ratio, liver to serum, is then 0.7 × total concentration in serum divided by the total concentration in the liver.

Table I—Binding of Warfarin in Rat Liver and Serum

Rat Number	Concentration, g or μg/ml		Free Fraction × 10^2		Free Fraction Ratio, Liver to Serum
	Liver	Serum	Liver	Serum	
1-I	0.977	0.288	0.417	2.02	0.206
2-I	0.976	0.590	0.614	1.45	0.423
3-I	0.764	0.244	0.422	1.89	0.223
4-I	1.31	0.517	0.365	1.32	0.277
5-I	0.863	0.494	0.469	1.17	0.401
6-I	0.933	0.408	0.306	1.00	0.306
7-I	0.895	0.313	0.358	1.46	0.245
8-I	0.897	0.297	0.121	0.523	0.231
9-I	0.851	0.491	0.183	0.455	0.402
10-I	1.07	0.369	0.0952	0.396	0.240
11-I	0.710	0.380	0.0980	0.261	0.375
12-I	1.13	0.393	0.0613	0.252	0.243
13-I	0.942	0.375	0.0632	0.227	0.278
Mean	0.948	0.397	0.275[a]	0.956[a]	0.296
SD	0.156	0.102	—	—	0.077

[a] The correlation between the liver and serum free fraction values is statistically significant ($r = 0.867$, $p < 0.001$).

Table II—Binding of Dicumarol in Rat Liver and Serum

Rat Number	Concentration, g or μg/ml		Free Fraction × 10^4		Free Fraction Ratio, Liver to Serum
	Liver	Serum	Liver	Serum	
1-II	2.36	7.78	3.47	1.50	2.31
2-II	3.22	7.60	5.61	3.39	1.65
3-II	2.59	9.08	5.22	2.13	2.45
4-II	4.37	9.08	5.41	3.72	1.45
5-II	2.79	7.85	12.3	6.25	1.97
6-II	3.86	7.35	5.06	3.79	1.34
7-II	3.35	8.58	13.9	7.79	1.78
8-II	4.91	7.38	8.33	7.90	1.05
19-II	3.81	5.24	5.71	5.94	0.961
10-II	4.88	6.88	7.21	7.30	0.988
Mean	3.61	7.68	7.22[a]	4.97[a]	1.59
SD	0.91	1.13	—	—	0.53

[a] The correlation between the liver and serum free fraction values is statistically significant ($r = 0.741$, $p < 0.02$).

RESULTS

The concentrations of warfarin in the liver and serum of 13 rats at the time when they were killed are listed in Table I. Also listed are the warfarin free fraction values in the liver and serum. The serum warfarin concentration was similar in all animals and averaged 0.397 ± 0.101 μg/ml (mean ± *SD*). Warfarin concentrations in the liver averaged about 2.5 times higher. The serum free fraction values ranged over almost one order of magnitude. The liver free fraction values ranged as widely, from 0.000613 to 0.00614, but were about 70% smaller than the free fraction values in serum. There is a statistically significant positive correlation between the liver and serum free fraction values.

The concentration and free fraction data for dicumarol are listed in Table II. The animals were killed when the dicumarol concentration in serum was 7.68 ± 1.13 μg/ml (mean ± *SD*). The concentration in the liver at that time was about one-half the concentration in serum. There was a fivefold range in serum free fraction values and a fourfold range in liver free fraction values. The liver and serum free fraction values show a statistically significant positive correlation.

Liver to serum and kidney to serum concentration ratios for warfarin were reported previously in tabular form (7) and are presented here in Fig. 1, plotted against total clearance. The same type of information for dicumarol (12) is presented in Fig. 2. In both cases, the liver to serum concentration ratios are considerably less variable than the free fraction values. There is no statistically significant correlation between the liver to serum concentration ratios and the serum free fraction values of either warfarin ($r = 0.27$, $p > 0.4$) or dicumarol ($r = 0.60$, $p > 0.1$).

The kidney to serum concentration ratios are considerably lower than the liver to serum concentration ratios and are also much less variable than the free fraction values. However, the kidney to serum concentration ratio of warfarin (Fig. 1) and the liver to serum concentration ratio of dicumarol (Fig. 2) increase slightly with increasing total clearance.

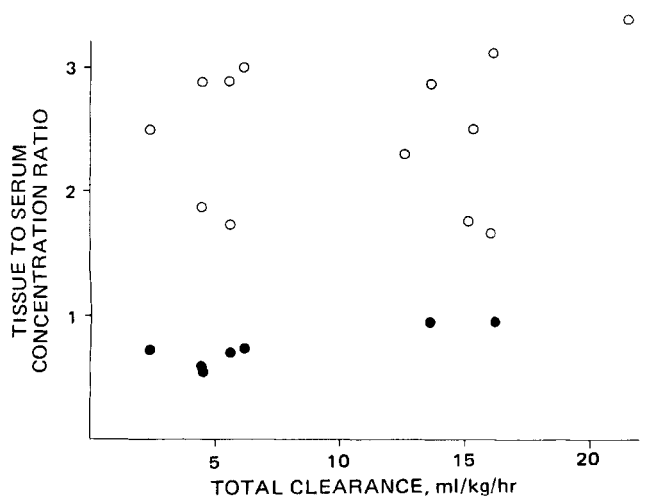

Figure 1—*Liver to serum* (○) *and kidney to serum* (●) *concentration ratios of warfarin in individual adult male rats, plotted as a function of the total clearance of the drug. Correlation coefficients are:* ○, r = *0.159* (p > *0.6*); *and* ●, r = *0.884* (p < *0.01*). *Kidney to serum concentration ratios were not determined in six animals.*

DISCUSSION

Almost one-half of the total amount of warfarin in the body is located in the liver (7). It is essential, therefore, to consider the binding of warfarin in the liver in the development of physiologically based pharmacokinetic models. The results of this study show that there are pronounced interindividual differences in the free fraction of warfarin in the liver. Interestingly, there is a positive correlation between liver and serum free fraction values. This causes the liver to serum concentration ratios of total (free and bound) warfarin to be relatively constant. Thus, these ratios are more suitable for pharmacokinetic modeling than are binding parameters derived from the free fraction values.

Before attempting a mechanistic explanation of the correlation of free fraction values in the liver and serum, the term "liver binding" should be amplified. Unlike plasma or serum, the liver cannot be viewed as an aqueous solution of proteins (plus various other components). Binding of drugs in the liver may involve some partitioning into lipoid phases and association with structural proteins that are hydrated but can hardly be considered to be in solution. In view of these complexities, the correlation of liver and serum free fraction values is helpful in that it permits some speculation concerning the reason(s) for the pronounced interindividual differences in these values.

These differences with respect to serum are not due to differences in the concentrations of albumin or total protein (7). They may be due to structural differences in the protein or (and this appears more likely) to qualitative and/or quantitative differences in endogenous inhibitors of

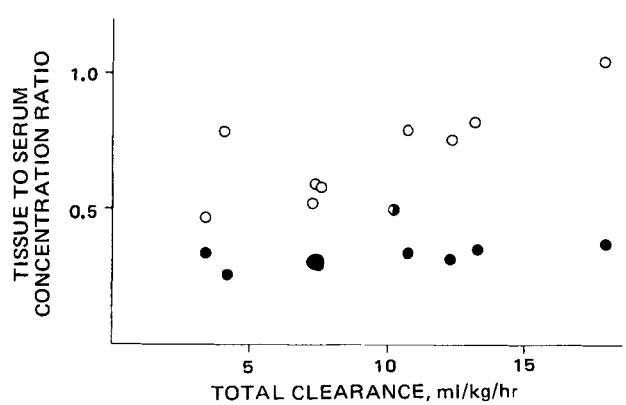

Figure 2—*Liver to serum* (○) *and kidney to serum* (●) *concentration ratios of dicumarol in individual adult male rats, plotted as a function of the total clearance of the drug. Correlation coefficients are:* ○, r = *0.718* (p < *0.02*); *and* ●, r = *0.425* (p > *0.2*).

protein binding. Such inhibitors exist in normal subjects and accumulate in patients with impaired or absent renal function (15, 16). It is not unreasonable to assume that these inhibitors compete with and, therefore, reduce the protein binding of certain drugs in serum as well as in tissues such as the liver. If that is so, a correlation of serum and liver free fraction values for a drug is to be expected.

The results obtained with dicumarol are consistent with those for warfarin: pronounced intersubject differences in liver free fraction values, a positive correlation of these values with the serum free fraction values, and, consequently, relatively little variation of liver to serum concentration ratios. It would be inappropriate to ascribe the lower liver to serum concentration ratios of dicumarol, as compared to those of warfarin, to the more extensive serum protein binding of the former. Obviously, tissue to serum concentration ratios depend on the relative binding of the drug in both phases.

In the case of dicumarol, there exists a pronounced concentration dependence of liver to serum concentration ratios at serum concentrations below about 7 μg/ml (3). At the lowest concentration studied, that ratio was about 5. The decrease in the liver to serum concentration ratio of dicumarol with increasing concentration (in the low serum concentration range) may be due to saturation of certain binding sites in the liver; it could also be a consequence of a cooperative effect of dicumarol binding on serum albumin at concentrations below 10 μg/ml (17, 18). Above that concentration, the serum free fraction of dicumarol remains essentially constant over a wide concentration range (11). Tissue to serum concentration ratios above unity can also occur if there exists an active "uphill" transport process from blood to the liver or within the liver. If that were the case, it would be impossible to calculate liver free fraction values as done here. It is unlikely that there would be a correlation between serum free fraction values and liver free fraction values if the latter were only apparent values, reflecting the kinetic parameters of a specialized transport process (unless, of course, the endogenous inhibitors presumed to be responsible for interindividual differences in free fraction values compete with warfarin and dicumarol for binding sites on serum proteins as well as for sites in the transport process).

The experimental studies required to resolve these frustrating uncertainties are very formidable and technically complex. However, until the resolution of these open questions, so-called physiologically based pharmacokinetic models for protein-bound drugs provide only limited capability for describing and predicting the characteristics of drug distribution processes in the body.

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Fenoprofen: Drug Form Selection and Preformulation Stability Studies

CLARENCE A. HIRSCH ˣ, RALPH J. MESSENGER, and JAMES L. BRANNON

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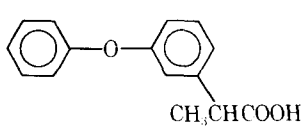
Abstract □ Several fenoprofen salts were prepared to obtain the most acceptable form for an oral dosage formulation. Thermal analysis techniques were used to compare stabilities of the water of hydration in different salt forms and to assess the effects of the water of hydration on compatibility with propoxyphene and codeine salts. Photodegradation products of fenoprofen were isolated and identified, and their relevance to product formulation was evaluated.

Keyphrases □ Fenoprofen—various salts synthesized, evaluated as oral dosage forms, stability studies □ Dosage forms, oral—various fenoprofen salts evaluated, stability studies □ Stability—various fenoprofen salts evaluated as oral dosage forms □ Anti-inflammatory agents—fenoprofen, various salts synthesized, evaluated as oral dosage forms, stability studies

Fenoprofen, (±)-α-methyl-3-phenoxybenzeneacetic acid (I), is a nonsteroidal, anti-inflammatory, analgesic, and antipyretic agent[1]. The pharmacology of fenoprofen was described previously (1), and absorption, metabolism, and excretion patterns in humans were reported (2, 3). Fenoprofen is safe and effective in the symptomatic treatment of rheumatoid arthritis (4–6) and is also useful for its analgesic (7) and antipyretic (8) effects.



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[1] Nalfon, fenoprofen calcium, developed by Lilly Research Laboratories.