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Comparative Pharmacokinetics of Coumarin Anticoagulants XXIV: Effect of Treatment with Phenobarbital on Serum Protein Binding of Warfarin and Dicumarol in Rats

AVRAHAM YACOBI, JOHN T. SLATTERY, and GERHARD LEVY [x]

Abstract □ Rats were treated with the enzyme inducer phenobarbital to determine if it would affect the serum protein binding of warfarin and dicumarol, possibly by changing the rate of formation or elimination of endogenous inhibitor(s). Daily administration of phenobarbital, 75 mg/kg, for 4 days increased relative liver size (a concomitant of enzyme induction) but had no apparent effect on the serum protein binding of warfarin and dicumarol.

Keyphrases □ Phenobarbital—effects on serum protein binding of warfarin and dicumarol, rats □ Protein binding, serum—warfarin and dicumarol, effect of phenobarbital, rats □ Binding, serum protein—warfarin and dicumarol, effect of phenobarbital, rats □ Warfarin—serum protein binding, effect of phenobarbital, rats □ Dicumarol—serum protein binding, effect of phenobarbital, rats □ Anticoagulants—warfarin and dicumarol, serum protein binding, effect of phenobarbital, rats □ Enzyme inducers—phenobarbital, effect on serum protein binding of warfarin and dicumarol, rats

The total clearance of warfarin by the body is directly proportional to the free fraction of this drug in serum of rats (1, 2) and humans (3). There are pronounced interindividual differences in free fraction values of warfarin (4) and corresponding differences in the total clearance of this drug by the body (2, 3). Ongoing studies in rats[1] indicate that this is true also for the other major coumarin anticoagulant, dicumarol. There is no relationship between the free fraction values of either warfarin (2) or dicumarol (data in this report) and the concentration of albumin in serum.

Recent studies showed that endogenous inhibitors in uremic as well as in normal human serum cause a decrease in the protein binding of warfarin and other drugs (5). Therefore, interindividual differences in the serum protein binding of warfarin and dicumarol may be caused by corresponding differences in the serum concentration of such inhibitors. Treatment with an enzyme inducer such as phenobarbital may change the serum protein binding of warfarin, dicumarol, and other drugs by changing the concentration of endogenous inhibitors in serum. This situation could result from a change in the formation or elimination kinetics of the inhibitors due to increased activity of certain enzyme systems. Accordingly, a study was initiated to determine the effect of treatment with phenobarbital on the protein binding of warfarin and dicumarol in the serum of rats.

EXPERIMENTAL

Forty-eight adult male Sprague–Dawley rats, ~350 g, were used for an initial screening study. About 3 ml of blood was obtained from the tail artery of each animal, and serum was separated. The free fraction of racemic warfarin in these serum samples was determined by equilibrium dialysis as described previously (2). Based on the results of this screening study, 20 rats were selected and classified into 10 pairs with very small intrapair and large interpair differences in the warfarin free fraction value.

Four days later, one member of each pair received phenobarbital, 75 mg/kg/day ip, for 4 days while the other member received injections of normal saline solution. One day later, the animals were sacrificed by removing all blood from the aorta under ether anesthesia. The liver was also removed, compressed slightly between paper tissue to remove remaining blood, and weighed. Serum was separated and used to determine the free fraction values for warfarin (2) and dicumarol (6) by equilibrium dialysis. The concentration of total protein in serum was determined by the method of Gornall *et al.* (7) with crystalline rat albumin as the standard, and the fraction of albumin was determined by electrophoresis using a commercial[2] serum protein electrophoresis system.

[1] To be published.

[2] Gelman Instrument Co., Ann Arbor, Mich.

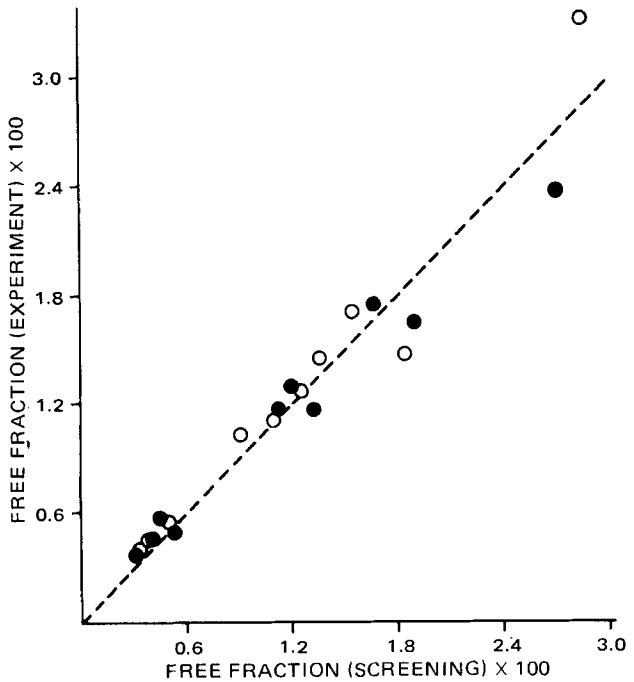

Figure 1—*Effect of phenobarbital treatment (75 mg/kg/day for 4 days) on the free fraction of warfarin in the serum of individual rats. Shown is a plot of free fraction values determined in a screening study preceding the experiment against the free fraction values in the same animals 1 day after the treatment period. Key: ●, animals treated with phenobarbital; and ○, control animals that received only saline solution. The stippled line has a slope of unity.*

RESULTS AND DISCUSSION

The warfarin free fraction value in the serum of the 20 selected rats ranged from 0.00344 to 0.0286 in the screening experiment. The percentage difference in the free fraction value within the 10 matched pairs was 3.9 ± 2.5 (mean ± *SD*). The correlation of free fraction values between the members of the 10 pairs was strong and highly statistically

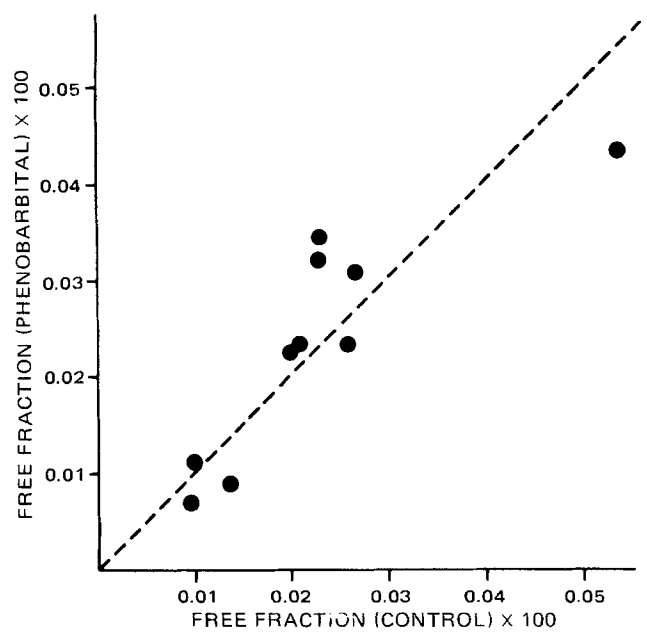

Figure 2—*Effect of phenobarbital treatment on the free fraction of dicumarol in the serum of rats. Plotted are the free fraction values in 10 control rats against the free fraction values in 10 phenobarbital-treated rats (r = 0.863, p < 0.001). The 10 pairs of rats were matched on the basis of nearly identical warfarin free fraction values in a preceding screening experiment. The stippled line has a slope of unity.*

Table I—Effect of Treatment with Phenobarbital on the Serum Protein Binding of Racemic Warfarin in Male Sprague–Dawley Rats

Group	Free Fraction × 100[a] Before	After	Ratio, After/Before[b]
Control	1.22 (0.344–2.86)	1.28 (0.389–3.34)	1.06 ± 0.107
Phenobarbital treated	1.17 (0.315–2.72)	1.14 (0.350–2.38)	1.03 ± 0.122[c]

[a] Mean (range) for 10 animals per group, 4 days before and 1 day after daily injections of phenobarbital (75 mg/kg) for 4 days. [b] Mean ± *SD*. [c] Not statistically significantly different from control ratio values.

significant ($r = 0.997$, $p < 0.001$). The group treated with phenobarbital had a significantly higher relative liver weight than the control group (37.0 ± 3.5 *versus* 30.9 ± 1.6 g/kg, $p < 0.001$), but there was no significant difference in serum albumin concentration (3.30 ± 0.20 *versus* 3.49 ± 0.20 g/100 ml, $p > 0.1$).

Treatment with phenobarbital had no apparent effect on the serum protein binding of warfarin (Table I). There was a strong correlation between the pretreatment and posttreatment free fraction values for individual animals (Fig. 1). The correlation coefficients for the data from control and phenobarbital-treated animals were 0.957 ($p < 0.001$) and 0.906 ($p < 0.001$), respectively.

It was not possible to obtain enough blood in the screening study to determine the free fraction values for dicumarol in addition to those for warfarin. However, in view of the previously documented strong correlation between the free fraction values for warfarin and dicumarol in individual rats (6), the warfarin free fraction values serve also to characterize and classify the animals with respect to serum protein binding of dicumarol. Therefore, it is possible to compare the posttreatment free fraction values for dicumarol in serum of phenobarbital-treated rats with the free fraction values of control animals. These values were 0.000238 (range of 0.000070–0.000432) for the phenobarbital group and 0.000225 (range of 0.000099–0.000540) for the control group and did not differ

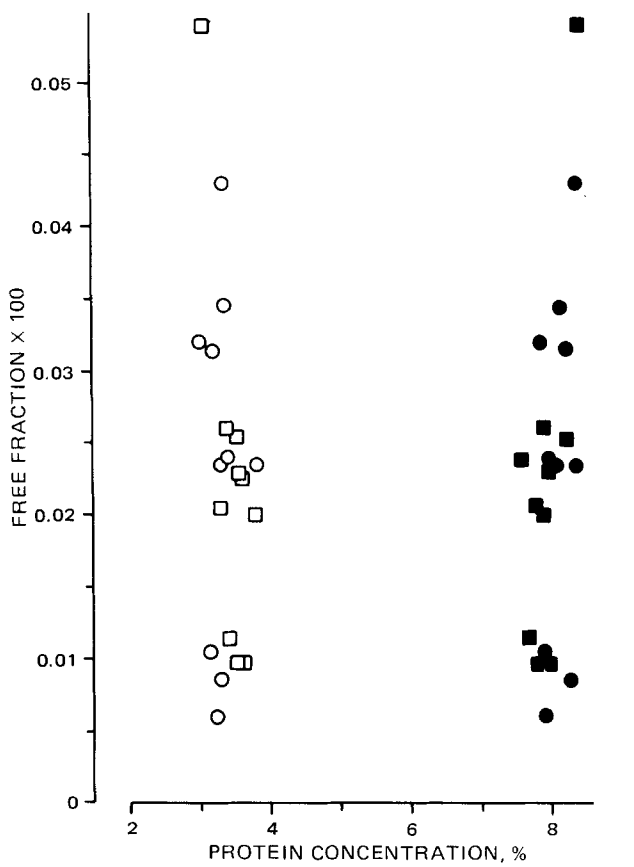

Figure 3—*Relationship between free fraction of dicumarol and the concentrations of albumin (open symbols) or total protein (closed symbols) in the serum of control rats (squares) and phenobarbital-treated rats (circles).*

significantly from one another. The ratio of free fraction values (treated/control) for the 10 matched pairs was 1.06 ± 0.27 (mean ± *SD*), and the correlation of these values between members of each pair was strong (Fig. 2).

Figure 3 is a plot of dicumarol free fraction values in serum against the serum concentration of albumin or total protein. The pronounced interindividual differences in serum protein binding of dicumarol were not related to corresponding differences in the concentration of serum proteins. Similar results were obtained previously for warfarin (2). Also consistent with previous observations (6) was the strong correlation between the free fraction values for dicumarol and warfarin in individual animals ($r = 0.937$, $p < 0.001$, $n = 20$ in this study).

The phenobarbital treatment regimen used is sufficient to cause pronounced enzyme induction, as reflected by the increased total clearance of warfarin[1] and dicumarol (8) and by the increase in relative liver weight. This treatment had no significant effect on the serum protein binding of warfarin and dicumarol. This result could mean that the concentrations of endogenous inhibitors in serum were not affected by enzyme induction or that the interindividual differences in serum protein binding of the coumarin anticoagulants are due to conformational differences of albumin, with enzyme induction having no effect on the steady-state concentration of that particular protein.

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Comparative Pharmacokinetics of Coumarin Anticoagulants XXV: Warfarin–Ibuprofen Interaction in Rats

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Abstract □ The effect of ibuprofen on the pharmacokinetics and anticoagulant action of racemic warfarin was determined in a crossover study on male Sprague–Dawley rats. At average plasma concentrations of 24–83 mg/liter, ibuprofen decreased the biological half-life and increased the total clearance of warfarin. It also increased the anticoagulant effect produced by a given plasma concentration of total (free and protein-bound) warfarin. These effects of ibuprofen appear to be a consequence of its displacing effect on warfarin in plasma.

Keyphrases □ Ibuprofen—effect on pharmacokinetics and anticoagulant action of warfarin, rats □ Pharmacokinetics—warfarin, effect of ibuprofen, rats □ Anticoagulant action—warfarin, effect of ibuprofen, rats □ Warfarin—pharmacokinetics and anticoagulant action, effect of ibuprofen, rats □ Anti-inflammatory agents—ibuprofen, effect on pharmacokinetics and anticoagulant action of warfarin, rats

The widely used nonsteroidal anti-inflammatory agent ibuprofen is a weak acid extensively bound to plasma proteins (1). As such, it may be expected to displace coumarin anticoagulants such as warfarin from plasma protein binding sites. This type of drug interaction can modify the distribution and elimination kinetics of warfarin and produce transient potentiation of its anticoagulant effect (2–4). Nevertheless, several clinical investigations failed to detect any effect of ibuprofen, in doses of up to 2.4 g/day, on the anticoagulant action of the coumarin drugs phenprocoumon and warfarin (5–8).

In view of the recent tendency to use doses of ibuprofen larger than 2.4 g/day for the treatment of inflammatory disease, it is important to determine *in principle* if ibuprofen interacts with warfarin under appropriate conditions (*i.e.*, at plasma ibuprofen concentrations sufficient to displace warfarin from protein binding sites). Such an investigation was carried out on rats dosed with ibuprofen at a rate producing ibuprofen concentrations moderately higher than those commonly encountered clinically.

EXPERIMENTAL

Male Sprague–Dawley rats[1], 250–300 g at screening time and 350–450 g during the interaction study, were maintained on a standard diet[2] with unrestricted access to water.

To determine the *in vitro* effect of ibuprofen on warfarin binding to rat serum protein, blood was obtained from the abdominal aorta of 11 ether-anesthetized rats and the serum was separated. To serum samples from individual rats were added 0.7–0.8 μg/ml of racemic ^{14}C-warfarin[3], 76 μCi/mg, and 0–150 μg/ml of racemic ibuprofen[4]. The free fraction of warfarin in serum (free/total warfarin concentration) was determined by equilibrium dialysis (9).

Sixty-three rats were screened for the *in vivo* interaction study by collecting 3 ml of blood from the tail artery under light ether anesthesia, separating the serum, and determining the free fraction of warfarin by equilibrium dialysis. Fourteen rats with widely differing warfarin free fraction values (0.0023–0.0157) were selected from this group for the

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Charles River Formula 4RF.
[3] Amersham/Searle Corp., Arlington Heights, Ill.
[4] Supplied by The Upjohn Co., Kalamazoo, Mich.