Comparative Pharmacokinetics of Coumarin Anticoagulants XLVI: Effect of Treatment with Phenobarbital on Pharmacokinetics of (S)-(−)-Warfarin in Rats

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Abstract □ This investigation was carried out (*a*) to determine if the enzyme inductive effect produced by phenobarbital reduces the interindividual variability in the biotransformation of a drug, as suggested in the literature; (*b*) to test whether the intrinsic clearance of free drug, for drugs exhibiting restrictive clearance, reflects the activity of drug-metabolizing enzyme systems; and (*c*) to determine if enzyme induction affects the apparent volume of distribution of a drug that tends to concentrate in the liver. Twelve pairs of adult male rats, matched with respect to their serum warfarin free fraction, received an intravenous injection of (*S*)-(−)-warfarin, 0.6 mg/kg, after four daily injections of either saline solution or phenobarbital (75 mg/kg). Phenobarbital treatment increased both the total and intrinsic clearance of (*S*)-(−)-warfarin almost threefold but did not reduce the coefficient of variation of the intrinsic clearance. Serum protein binding of (*S*)-(−)-warfarin was not affected by phenobarbital treatment. The biological half-life of warfarin and the duration of its anticoagulant effect were reduced substantially by treatment with phenobarbital. Consistent with pharmacokinetic theory, the relationship between total clearance and the free fraction of warfarin in serum remained approximately linear, but the slope of the regression line was increased for the animals treated with phenobarbital.

Keyphrases □ Warfarin—pharmacokinetics, effect of phenobarbital on drug biotransformation and enzyme induction, rats □ Pharmacokinetics—warfarin biotransformation, inductive enzyme effect of phenobarbital, rats □ Phenobarbital—effect on enzyme induction, effect on warfarin biotransformation, pharmacokinetics, intersubject variability determination, rats □ Anticoagulants—warfarin, effect of phenobarbital on warfarin biotransformation, rats

It is well known that treatment with phenobarbital enhances the elimination of warfarin in both humans and rats (1–7). *In vitro* studies indicated that phenobarbital causes induction of the enzyme system(s) responsible for the biotransformation of warfarin (6), but these studies failed to test for variability in the degree of induction (as measured by the enhancement of warfarin elimination) as a function of the elimination kinetics of warfarin prior to enzyme induction. This question is important because there are pronounced interindividual differences in the elimination kinetics of warfarin in humans as well as in animals (8, 9).

BACKGROUND

Intersubject variability in antipyrine half-life decreased when subjects were treated with phenobarbital, and it was suggested that treatment with phenobarbital (or another enzyme inducer) might have a similar effect on the variability in the elimination kinetics of other drugs (10). In the cited study, the relative decrease in the half-life of antipyrine after phenobarbital administration was proportional to the prephenobarbital half-life.

Previous reports in this series showed that the total body clearance of warfarin is highly dependent on the serum protein binding of the drug, specifically on its free fraction in serum (9, 11). Recent studies revealed a 15-fold range in the serum free fraction of warfarin in rats (8, 12) and an approximately fourfold variation in humans (9, 13–15).

The proportionality constant between the total clearance and the free fraction for nonperfusion rate-limited systems is the intrinsic clearance k''. Thus, $k'' = TC/f_p$, where TC is the total body clearance and f_p is the free fraction of drug in serum or plasma (11). An essentially linear relationship between TC and f_p of warfarin has been observed in both humans and rats (9, 11). This relationship permits isolation of the effects of certain treatments or procedures on the protein binding of a drug from the effects on the activity of enzymes responsible for the biotransformation of that drug. This ability should help to determine the reasons for a change in the total clearance of a drug.

If enzyme induction leads to a decrease of the intersubject variability in the biotransformation kinetics of a drug, then a decrease in the coefficient of variation of k'' should be observed. Furthermore, if the relative degree of enhancement of drug elimination caused by induction of an enzyme system is proportional to the preinduction activity of the enzyme system, then the relative increase in intrinsic clearance caused by induction should be proportional to its preinduction (control) value.

Treatment of rats with phenobarbital produces an increase in their relative liver weight (16). Since the liver contains a large fraction of the amount of warfarin in the body of a rat (8, 17), treatment of rats with phenobarbital might increase the apparent volume of distribution of warfarin. This possibility has not been explored to date.

The purposes of this investigation were (*a*) to determine if the enzyme inductive effect produced by phenobarbital reduces the interindividual variability in the biotransformation of (*S*)-(−)-warfarin; (*b*) to test whether the intrinsic clearance of free (*S*)-(−)-warfarin reflects the activity of drug-metabolizing enzyme systems; and (*c*) to determine if enzyme induction affects the apparent volume of distribution of (*S*)-(−)-warfarin. (*S*)-(−)-Warfarin rather than racemic warfarin was used to decrease the complexity of the experiment since the metabolism of the individual enantiomers might be induced to a different extent. Since the (*S*)-(−)-enantiomer of warfarin is a more potent anticoagulant and has a longer biological half-life than the (*R*)-(+)-enantiomer in rats (18), experiments with (*S*)-(−)-warfarin are easier to perform.

EXPERIMENTAL

Seventy-two adult male Sprague–Dawley rats[1], 250–300 g initially and 320–400 g during the study, were screened to obtain two groups of rats matched individually with respect to their free fraction of warfarin in serum.

For screening purposes, 3 ml of blood was taken from the tail artery of the rats and the serum was separated. Approximately 1 μg/ml of racemic [^{14}C]warfarin[2] (specific activity 76 μCi/mg) was added to the serum, and its free fraction was determined by equilibrium dialysis for 20 hr against isotonic pH 7.4 phosphate buffer at 37° (8). Twenty-four rats were paired according to their free fraction of warfarin in serum. One member of each pair was assigned to the group to be treated with phenobarbital, and the other served as a control. The free fraction values ranged from 0.00296 to 0.0158.

Two weeks after the screening experiment, the rats in the group designated for phenobarbital treatment received 75 mg of phenobarbital/kg ip daily for 4 days, while the control group received injections of a comparable volume of normal saline for the same period. The rats had unrestricted access to food[3] and water throughout the study.

On Day 5, the rats in both groups received a single dose of [^{3}H](*S*)-(−)-warfarin[4] (0.578 mg/kg, specific activity 0.943 mCi/mg, in isotonic

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Amersham-Searle Corp., Arlington Heights, Ill.
[3] Charles River formula 4RF.
[4] Research Triangle Inc., Research Triangle Park, N.C.

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Table I—Effect of Phenobarbital on Pharmacokinetics of (*S*)-(−)-Warfarin in Matched Pairs of Rats [a]

Parameter	Control	Phenobarbital	Ratio of Phenobarbital to Control
Serum free fraction × 100	0.266–1.29[b]	0.315–1.42	1.04 ± 0.14[b]
Half-life, hr	10.6–47.2	3.28–30.1	0.409 ± 0.114
Volume of distribution, ml/kg	103–155	112–185	1.13 ± 0.08
Total clearance, ml/(hr kg)	1.56–9.62	2.58–35.5	2.95 ± 0.78
Intrinsic clearance of free drug, [ml/(hr kg)] × 100	7.00 ± 1.65[b]	19.9 ± 5.07[c]	2.85 ± 0.80[b]
Duration of anticoagulant effect[d], hr	62–124	25–75	0.469 ± 0.079

[a] Twelve pairs of rats matched with respect to their serum free fraction of racemic warfarin. Results are shown as ranges of values for those parameters that are not normally distributed due to the intentional selection of animals with a wide range of free fraction values. Ratio and intrinsic clearance data are reported as the mean ± *SD*. [b] Data from 11 animals or animal pairs only due to loss of the blood sample for the serum free fraction determination from one rat. [c] Statistically significantly different from control (*t* test, $p < 0.001$). [d] Time required for prothrombin complex activity to return to 60% of normal after intravenous injection of 0.6 mg/kg.

saline adjusted to pH 7.4) *via* the tail vein. Blood samples (0.27 ml) were obtained at ~2–6-hr intervals from the tail artery (19). The plasma was separated, and the warfarin concentration in 0.1 ml of plasma was determined by TLC and scintillation counting as described previously (8, 20). Tritium exchange has been shown to be negligible under these experimental conditions (21). Prothrombin complex activity in plasma was measured (19) to determine the duration of the anticoagulant effect.

At the completion of the study, the rats were sacrificed by removing as much blood as possible from the abdominal aorta under ether anesthesia. The serum was separated, 0.5 μg/ml of [^{3}H](*S*)-(−)-warfarin (specific activity 3.77 mCi/mg) was added, and the free fraction was determined by equilibrium dialysis as described. The total intrinsic clearances, half-life, and apparent volume of distribution were determined as described previously (18, 20).

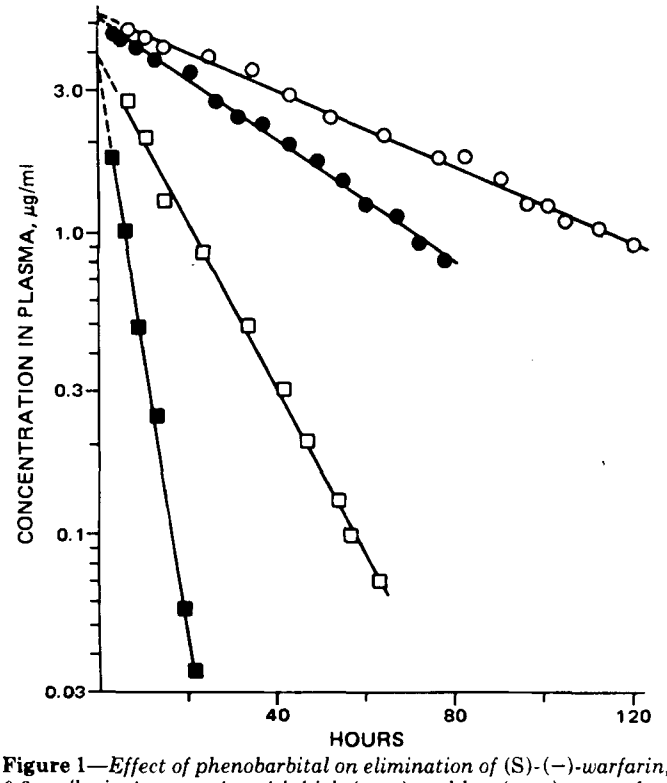

Figure 1—*Effect of phenobarbital on elimination of* (S)-(−)-*warfarin, 0.6 mg/kg iv, in rat pairs with high* (□,■) *and low* (○,●) *serum free fraction values of the drug. Key: solid symbols, phenobarbital-treated animals; and open symbols, control animals.*

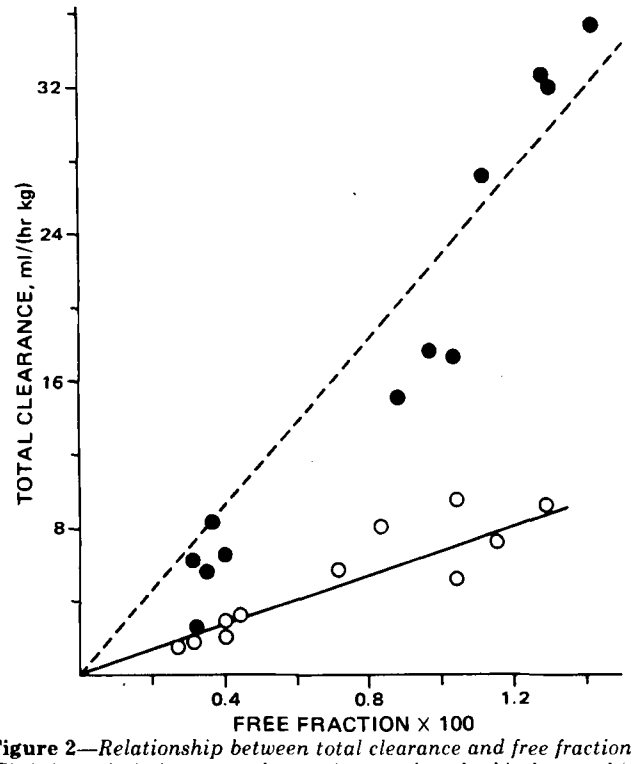

Figure 2—*Relationship between total clearance and free fraction of* (S)-(−)-*warfarin in serum of rats. Key:* ●, *phenobarbital treated* ($r = 0.968$, $p < 0.001$); *and* ○, *control* ($r = 0.902$, $p < 0.001$). *Regression lines were forced through the origin.*

RESULTS

The initial screening study permitted the selection of well-matched pairs of rats over a wide range of serum racemic warfarin free fraction values. The mean ± *SD* of the free fraction ratios of the 12 pairs was 1.00 ± 0.05. The correlation coefficient of the free fraction values for the two members of each pair in the entire group was 0.995 ($p < 0.001$).

In agreement with previous observations (18), the free fraction value of racemic warfarin determined in the screening study correlated strongly with that of the free fraction of (*S*)-(−)-warfarin determined at the end of the experiment ($r = 0.982$, $p < 0.001$), indicating the suitability of the racemic warfarin free fraction for screening purposes. The ratio of racemic to (*S*)-(−)-warfarin free fraction values (mean ± *SD*) was 1.12 ± 0.068 for the control rats and 1.10 ± 0.18 for the phenobarbital-treated rats, showing that phenobarbital treatment had no apparent effect on the free fraction of warfarin in serum. This lack of effect is consistent with previous observations (22).

The similarity of warfarin free fraction values in the rat pairs was maintained throughout the study (Table I). The correlation coefficient of the free fraction value of (*S*)-(−)-warfarin within pairs at the end of the study was 0.984 ($p < 0.001$).

The time course of the (*S*)-(−)-warfarin concentration in serum after intravenous injection in individual members of two pairs of rats (those with the highest and lowest mean free fraction values, respectively) is shown in Fig. 1. A summary of the pharmacokinetic data obtained in the study is presented in Table I. The biological half-life of (*S*)-(−)-warfarin and the duration of the anticoagulant effect in rats treated with phenobarbital were ~ 50–60% shorter on the average than those of the control member of each pair. On the other hand, the apparent volume of distribution showed no statistically significant change by the Wilcoxon test, even though it was larger in 11 of 12 phenobarbital-treated animals than in their controls. A strong correlation was observed in both groups between the first-order elimination rate constant and the free fraction of (*S*)-(−)-warfarin in serum ($r = 0.983$ and $r = 0.938$ for phenobarbital-treated and control groups, respectively; $p < 0.001$ for both groups). As observed previously (8), the apparent volume of distribution also correlated with the free fraction of warfarin in serum based on combined results from both groups ($r = 0.713$, $p < 0.001$).

On the average, the total clearance of (*S*)-(−)-warfarin was almost three times higher in rats treated with phenobarbital than in their respective controls (Table I). The intrinsic clearance in the phenobarbital-treated rats also was approximately three times higher on the average than that

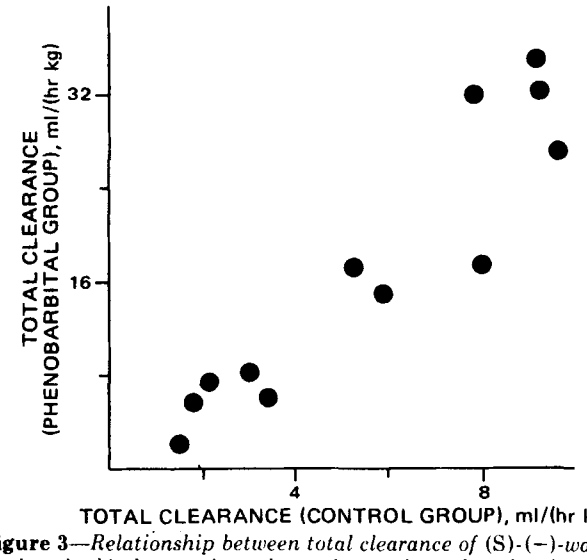

Figure 3—*Relationship between total clearance of* (S)-(−)-*warfarin in phenobarbital-treated member and control member of each matched pair of rats* ($r = 0.931$, $p < 0.001$).

of the control member of each pair. Treatment with phenobarbital did not decrease the coefficient of variation of the intrinsic clearance; it was 24% in the control group and 26% in the phenobarbital group.

The relationship between the free fraction of (*S*)-(−)-warfarin in serum and the total clearance for control and phenobarbital-treated animals is shown in Fig. 2. Essentially linear relationships were obtained in each case. The slope of a plot of the relative increase (expressed in percent) in the intrinsic clearance caused by phenobarbital treatment (comparing members of matched rat pairs) *versus* the control intrinsic clearance was not significantly different from zero. There was a strong and apparently linear relationship between the total clearance of (*S*)-(−)-warfarin in the control member and phenobarbital-treated member of each rat pair (Fig. 3).

DISCUSSION

The dose and the duration of phenobarbital administration used in this study were sufficient to produce a pronounced enzyme inductive effect as reflected by an almost threefold increase in the intrinsic clearance of (*S*)-(−)-warfarin. However, enzyme induction did not decrease the interindividual variability of the intrinsic clearance; there was essentially no difference in the coefficient of variation of the intrinsic clearance between the control and treated animals. Since phenobarbital treatment had no effect on the serum protein binding of (*S*)-(−)-warfarin, and since the relative effect of enzyme induction was independent of the normal intrinsic clearance value, the relationship between the total clearance and the serum free fraction remained essentially linear as predicted by theory (11). Thus, the slope of such a plot, *i.e.*, the intrinsic clearance of free drug, does reflect the effect of enzyme induction.

In a recent study, it was found that the liver contains about 44% of the total amount of warfarin in normal (not phenobarbital-treated) rats (8). The phenobarbital treatment regimen used in this study increased the relative liver weight of rats by ~22% (16). This increase suggests that the apparent volume of distribution of warfarin should increase by ~10% in phenobarbital-treated rats. The results of the present study show that the apparent volume of distribution of (*S*)-(−)-warfarin was increased by 13% on the average, but this increase was not statistically significant. Statistical analysis of the data for the probability of a type II error strongly suggests ($P_B \simeq 0.4$) that a real difference of 10% would not be detected in our study at $\alpha = 0.05$ (23).

Branch and Shand (24) recently reevaluated previously published data on the effect of enzyme induction on antipyrine elimination kinetics in humans. Rather than basing their analysis on the biological half-life as did Vesell and Page (10), they used the clearance value as a more appropriate index of the activity of antipyrine-metabolizing enzyme systems. They found that the intersubject variability of antipyrine clearance by nine normal subjects was as large after phenobarbital treatment as in the noninduced state. The results of the present study with warfarin are consistent with these conclusions and are particularly noteworthy because the degree of enzyme induction was more pronounced than in the antipyrine study.

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