

Stereoselective Interaction of Phenylbutazone with $[^{12}\text{C}/^{13}\text{C}]$ Warfarin Pseudoracemates in Man

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Research Article

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Stereoselective Interaction of Phenylbutazone with [$^{12}\text{C}/^{13}\text{C}$]Warfarin Pseudoracemates in Man

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ABSTRACT To evaluate the interaction of phenylbutazone with racemic warfarin or *R,S*-(\pm)-warfarin in man, *S*-($-$)-warfarin or levowarfarin was synthesized with ^{13}C label in the 2-position of the coumarin nucleus and added to [^{12}C]*R*(+)-warfarin or dextrowarfarin to form a [$^{12}\text{C}/^{13}\text{C}$]pseudoracemate of warfarin. In six normal human subjects, a single oral dose of this "cold labeled" pseudoracemate, 1.5 mg/kg body weight, was administered with and without a daily dosage of phenylbutazone, 300 mg orally, beginning 3 d before the warfarin dose and continuing throughout the hypoprothrombinemia. Plasma samples were obtained daily and analyzed for warfarin content and for one-stage prothrombin activity. Unchanged warfarin in the plasma was fractionated by normal-phase, high-pressure liquid chromatography, and the enantiomeric ratios were determined by chemical-ionization mass spectrometry with pentadeuteriowarfarin as the internal standard. A highly significant augmentation of the hypoprothrombinemia of the pseudoracemate occurred during the phenylbutazone regimen ($P < 0.001$) compared with pseudoracemic warfarin administered alone. There was a highly significant increase in the plasma clearance of dextrowarfarin ($P < 0.01$) and a significant decrease in the plasma clearance of levowarfarin ($P < 0.05$) during the phenylbutazone regimen compared with administration of warfarin alone. It was concluded that phenylbutazone augmented the hypoprothrombinemia of pseudoracemic warfarin stereoselectively by inhibiting the metabolic disposition of the more hypoprothrombinemic levowarfarin, yet reduced the plasma levels of pseudo-

racemic warfarin by greatly augmenting the metabolic disposition of dextrowarfarin.

INTRODUCTION

The interaction of phenylbutazone and racemic warfarin in man is paradoxical in that the augmentation of the anticoagulant effect is associated with a more rapid elimination of total warfarin (1). This effect was said to result from displacement of the warfarin in plasma from its albumin-binding sites by the more highly bound phenylbutazone resulting in an increased fraction of free warfarin. Lewis et al. (2) reported a differential effect of phenylbutazone on the enantiomorphs of racemic warfarin, *S*-($-$)-warfarin (levowarfarin) and *R*-(+)-warfarin (dextrowarfarin) (2). They found that phenylbutazone decreased the clearance from plasma of levowarfarin, the more potent anticoagulant agent, and markedly increased the clearance of dextrowarfarin. Another study in man on the interaction of phenylbutazone with the separated enantiomorphs showed augmentation of the hypoprothrombinemia and an increase in plasma concentrations of levowarfarin, no significant alteration of the hypoprothrombinemia and a marked decrease in plasma concentrations of dextrowarfarin (3). To determine simultaneously the fate of both enantiomorphs of racemic warfarin, the form used clinically, levowarfarin was synthesized with a stable isotope of carbon, ^{13}C , in the 2-position of the coumarin nucleus (4). By combining the [^{13}C]levowarfarin with an equal amount of common [^{12}C]dextrowarfarin, a [$^{12}\text{C}/^{13}\text{C}$]warfarin pseudoracemate was prepared (5). This preparation was administered to normal human subjects to study the simultaneous interaction of phenylbutazone with each enantiomorph of racemic warfarin.

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METHODS

Subjects. Six male subjects, 18–30 yr old, were studied. All were paid volunteers who were carefully informed of the nature of the experiments and who signed written consents in accordance with all the conditions required by federal regulation and the local Research and Human Subject Review Committee. All were in excellent health and had not taken any other drug during the preceding 2 mo. Each subject served as his own control and the order of the experiments with warfarin alone and with warfarin plus phenylbutazone in the crossover studies was assigned randomly. The data were analyzed by Student's *t* test for paired observations; *P* values were determined from probability tables for one-tailed tests (6).

Measurement of anticoagulant effect. The one-stage prothrombin activity was determined by the method of Quick, as described previously (7), in which the test result in seconds was converted to percentage of prothrombin activity by a saline-dilution curve of pooled normal plasma. The total hypoprothrombinemic effect for each experiment during the control period in which warfarin was administered alone was determined by measuring the total area under the curve (AUC)¹ for the one-stage prothrombin time expressed in seconds on a semilogarithmic scale by the trapezoidal rule and was expressed in arbitrary units (7). The increase in AUC-prothrombin time for each subject was determined for the experimental period in which pseudoracemic warfarin plus phenylbutazone were administered.

Synthesis and preparation of [¹²C/¹³C]pseudoracemate of warfarin. (S)-warfarin containing 90% enriched ¹³C exclusively in the C2 position was synthesized as described separately (4). The pseudoracemic mixture was prepared by recrystallization from an acetone solution containing 1.0 g each of ¹³C (S)-warfarin -C2- (90%) and (R)-warfarin. The purity of the crystalline material obtained was established by analysis on high-pressure liquid chromatography (HPLC), five different thin-layer chromatographic systems, and by chemical ionization mass spectrometry. Gelatin capsules with a 200-mg capacity, containing either 1, 2, 5, 10, 25, or 50 mg of pseudoracemic warfarin in a lactose (U. S. Pharmacopeia) matrix were prepared for administration. The biologic authenticity of the pseudoracemate as powder in capsules was verified by comparing its hypoprothrombinemic effect and its blood levels to that of true racemic warfarin as tablets in these same six subjects. For racemic and pseudoracemic warfarin, the mean ± SEM for AUC-prothrombin time were 90 ± 12 and 85 ± 10 U, respectively, an insignificant difference (*t*, 0.68, *P* > 0.4). The blood levels measured as AUC-warfarin were 616 ± 63 and 630 ± 34 mg/liter × h for racemic and pseudoracemic warfarin, also an insignificant difference (*t*, 0.40, *P* > 0.4).

Preparation of plasma specimens for measurement of warfarin. Details of the analytical methodology have been reported separately (5). A brief description of the method follows. Serial plasma samples, 2 ml, were spiked with 10 μg of the internal standard, pentaphenyldeuteriowarfarin, acidified with hydrochloric acid and extracted with 1,2-dichloroethane. The organic layer was extracted with 2.5 N sodium hydroxide and discarded. The basic aqueous layer then was acidified with hydrochloric acid and extracted with 1,2-dichloroethane. An aliquot of the organic layer was transferred to a liquid scintillation vial and evaporated to

dryness. The residue was taken up into mobile phase consisting of cyclohexane:isopropanol:glacial acetic acid, in a ratio of 96:3.95:0.05 and injected onto a HPLC column. The HPLC column used for separation was packed with DuPont 5-μm Zorbax-CN column material (E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.). Unchanged racemic warfarin had a retention time of ~9 min and was detected with a 280-nm fixed wavelength detector. Phenylbutazone emerged shortly after the solvent front. The unchanged racemic warfarin peak was collected in a scintillation vial and evaporated to dryness. A second pass through the HPLC gave unchanged racemic warfarin free from all contamination with phenylbutazone. The racemic warfarin peak was collected and the solvent evaporated to dryness.

Mass spectrometric determination of enantiomorphous ratios and absolute amounts of warfarin enantiomorphs. Details of the mass spectral analytical procedure have been reported separately (5). A brief description of the method follows. The HPLC-separated pseudoracemic warfarin residues were reconstituted in 100 μl of acetonitrile. The solution was applied to the probe tip of the direct insertion probe, evaporated, and inserted into the mass spectrometer. Mass spectral peaks at 309 (MH⁺ ion of [R]-warfarin), 310 (MH⁺ ion of [S]-warfarin-C2-¹³C [90%]), and 314 (MH⁺ ion of pentaphenyldeuteriowarfarin) were integrated under computer-controlled selected ion monitoring. The ratio of 309:310 provided a measure of R,S-warfarin, whereas the ratio of 309 + 310:314 provided a measure of the absolute amount of pseudoracemic warfarin present in the biological sample. Standard curves for both measurements were accurate to <5% over the range of concentrations determined.

Experiments. Each subject received pseudoracemic warfarin acid, 1.5 mg/kg body wt, in 200-mg capsules containing 1–50 mg of pseudoracemic warfarin acid to equal 1.5 mg/kg of drug. Each subject swallowed all the capsules allotted to him with a full glass of water in the morning at least 2 h after breakfast; no food was ingested for at least 2 h thereafter. Blood samples were obtained by venipuncture at 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168, 192, 216, and 240 h after the administration of pseudoracemic warfarin. The blood was mixed in glass tubes in a proportion of 9:1 with a combination of three parts 0.1 M sodium citrate and two parts 0.1 M citric acid, and centrifuged at 2,500 rpm for 30 min at 4°C. The plasma was removed and stored in several small glass tubes at –20°C.

After a 4-wk rest period, the same subject received 1 100-mg tablet of phenylbutazone (Butazolidin; Geigy Pharmaceuticals, Ardsley, N. Y.), 3 times/d: before breakfast, before dinner, and at bedtime. On the 4th d of the phenylbutazone regimen, each subject received the same dose of pseudoracemic warfarin as before; 1.5 mg/kg body wt. The daily regimen of phenylbutazone was continued for the duration of the hypoprothrombinemia, 6–12 d, and then was discontinued. Venous blood samples were obtained at the same times as during the control period of pseudoracemic warfarin alone and were processed in the same manner. No side effects from either drug were observed in any subject.

Each subject also received a single dose of truly racemic warfarin sodium (Coumadin sodium; Endo Laboratories, Inc., Garden City, N. Y.) alone, 1.5 mg/kg orally as tablets. Daily blood samples were obtained to determine the blood concentrations of racemic warfarin and the prothrombin-time responses for each subject for direct comparison of these parameters with those obtained with pseudoracemic warfarin acid.

Calculations. The half-life of the terminal elimination phase of the log plasma warfarin from 12 to 144 h was cal-

¹Abbreviations used in this paper: AUC, area under the curve; HPLC, high-pressure liquid chromatography; Vd, volume of distribution.

culated by the method of least squares. The slope of the terminal phase, β , was calculated from the quotient of the factor 0.693 and the half-life for each subject. The plasma concentration of warfarin at time zero, C_p^0 , was calculated by extension of the least squares line for half-life to time zero. The apparent volume of distribution, V_d , was calculated from the quotient of the dose of warfarin and plasma concentration at time zero for each subject. The area under the curve for the plasma concentrations of each enantiomorph of warfarin, AUC-warfarin, was determined from time 0 to 240 h by the trapezoidal rule. The plasma clearance of each enantiomorph of warfarin for each subject was calculated from the mean of the product of V_d and β and from the quotient of the warfarin dose and AUC-warfarin.

RESULTS

[^{12}C] $R(+)$ -warfarin. After administration of the warfarin pseudoracemate alone, all subjects had detectable concentrations of dextrowarfarin in their plasma at 1 h (Fig. 1). The highest concentration of dextrowarfarin was achieved at 2 h in one subject, at 4 h in three subjects, at 8 h in one subject, and at 12 h in one subject.² The highest mean concentration of dextrowarfarin in plasma occurred at 4 h. When the warfarin pseudoracemate was administered during the phenylbutazone regimen, all of the subjects had detectable concentrations of dextrowarfarin in plasma at 1 h and had the highest mean concentration at 8 h. The highest concentration of dextrowarfarin during the phenylbutazone regimen was achieved at 2 h in one subject, at 4 h in one subject, at 8 h in three subjects, and at 12 h in one subject. The highest mean concentration of dextrowarfarin in plasma during the phenylbutazone regimen occurred at 8 h, compared with 4 h for dextrowarfarin alone. The mean concentration of dextrowarfarin during the phenylbutazone regimen compared with dextrowarfarin alone was significantly lower at 1 h ($P < 0.05$) and 2 h ($P < 0.03$). Thus, the phenylbutazone regimen resulted in a slower rate of absorption of dextrowarfarin from the pseudoracemate.

After the peak concentrations were reached, the plasma concentrations of dextrowarfarin declined by apparent first-order kinetics in all subjects for the duration of the experiments both with and without phenylbutazone (Fig. 1). The mean concentrations of dextrowarfarin with and without phenylbutazone from 4 to 12 h were not significantly different. They were significantly lower ($P < 0.01$) from 24 to 168 h and at 216 h during the phenylbutazone regimen and at 192 and 240 h ($P < 0.05$). The AUC-warfarin for dextrowarfarin during the phenylbutazone regimen was reduced in all subjects. The mean AUC-warfarin for dextrowarfarin during the phenylbutazone regimen, compared with that for dextrowarfarin alone,

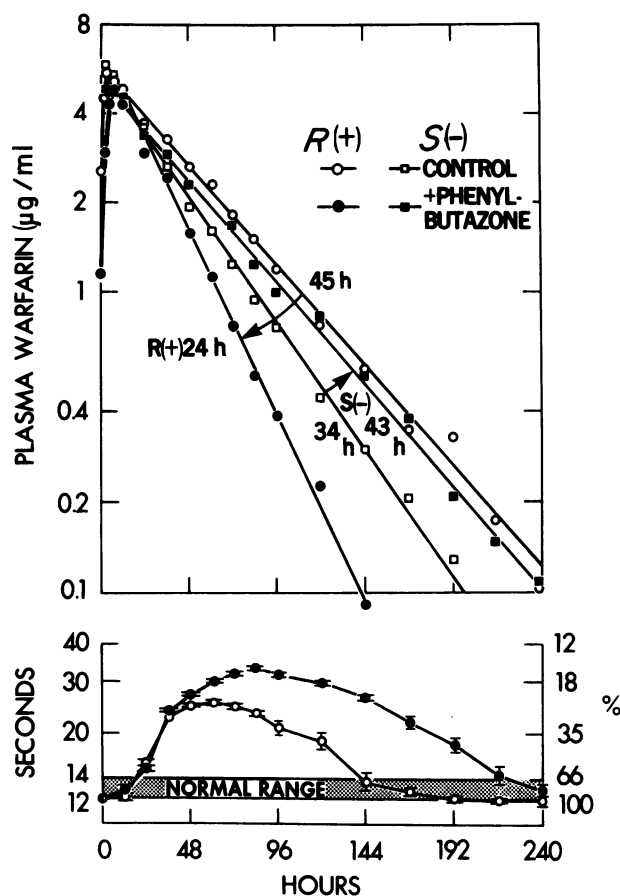


FIGURE 1 Blood levels of warfarin and one-stage prothrombin times after single oral doses of [$^{12}\text{C}/^{13}\text{C}$]pseudoracemic warfarin in six normal subjects. Phenylbutazone, 300 mg daily by mouth, was administered for 3 d before the pseudoracemic warfarin dose and continued for the duration of the hypoprothrombinemia. The lines for half-life were computed by the method of least squares. On the lower graph the mean \pm SEM of the one-stage prothrombin test is expressed on the left ordinate in seconds logarithmically and on the right ordinate in percent of normal activity. Phenylbutazone markedly shortened the half-life of $R(+)$ warfarin, prolonged the half-life of $S(-)$ warfarin, and markedly augmented the hypoprothrombinemia of the pseudoracemate.

was reduced to a highly significant degree ($P < 0.002$) (Table I).

[^{13}C] $S(-)$ -warfarin. When the warfarin pseudoracemate was administered alone, all subjects had detectable concentrations of levowarfarin at 1 h (Fig. 1). The highest concentration of levowarfarin was achieved at 2 h after the administration of warfarin in one subject, at 4 h in three subjects, at 8 h in one subject, and at 12 h in one subject. The highest mean concentration of levowarfarin in plasma occurred at 4 h. When the warfarin pseudoracemate was administered during the phenylbutazone regimen, all of the subjects had detectable concen-

² Extensive tables of the individual data are available from the authors upon request.

TABLE I
Pharmacokinetic Analysis of Plasma Concentration of [^{12}C]R(+)-Warfarin and [^{13}C]S(-)-Warfarin after Single Dose of Warfarin with and without Phenylbutazone*

Drug regimen	Cp ⁰	Vd	t _{1/2}	β	AUC _w	Q
	mg/liter	liter	h	h ⁻¹	mg/liter \times h	ml/min
[^{12}C]R(+)-warfarin						
Warfarin alone	6.38 \pm 0.39	9.0 \pm 0.5	41.2 \pm 1.7	0.017 \pm 0.001	354 \pm 20	2.61 \pm 0.14
Plus phenylbutazone	6.59 \pm 0.43†	8.8 \pm 0.7	26.4 \pm 1.6§	0.027 \pm 0.002	210 \pm 19	4.25 \pm 0.32
[^{13}C]S(-)-warfarin						
Warfarin alone	7.44 \pm 0.60	7.8 \pm 0.6	30.5 \pm 5.7	0.026 \pm 0.004	277 \pm 33	3.52 \pm 0.46
Plus phenylbutazone	6.13 \pm 0.24¶	9.3 \pm 0.4	40.0 \pm 3.8¶	0.018 \pm 0.002¶	327 \pm 25¶	2.86 \pm 0.24¶
[^{12}C / ^{13}C]R,S(\pm)-warfarin						
Warfarin alone	13.05 \pm 0.75	8.8 \pm 0.5	36.2 \pm 3.2	0.020 \pm 0.002	630 \pm 34	2.96 \pm 0.26
Plus phenylbutazone	12.29 \pm 0.43	9.2 \pm 0.3	33.9 \pm 1.8	0.017 \pm 0.001	528 \pm 41	3.43 \pm 0.27¶

Abbreviations used in this table: Cp⁰, plasma concentration of warfarin at time zero, calculated by extrapolation of β ; Vd, apparent volume of distribution, calculated from dose of warfarin divided by Cp⁰; t_{1/2}, half-life of warfarin concentrations calculated by least squares method; β , slope of the terminal phase of plasma concentrations of warfarin; AUC_w, total area under curve for plasma concentrations of warfarin; Q, warfarin clearance calculated from the mean of the product of Vd and β and of the quotient of the warfarin dose divided by AUC_w.

* Warfarin pseudoracemate, 1.5 mg/kg orally, composed of 0.75 mg/kg each of [^{12}C]R(+)-warfarin and of [^{13}C]S(-)-warfarin; phenylbutazone, 100 mg orally three times daily beginning 3 d before the warfarin dose and continuing 10 d after it. Mean body weight of the six subjects was 75.5 kg.

† *t* test for paired observations.

§ *P* < 0.001.

^{||} *P* < 0.01.

¶ *P* < 0.05.

trations of levowarfarin in plasma at 1 h and had the highest mean concentrations at 8 h. The mean concentration of levowarfarin during the phenylbutazone regimen compared with that during warfarin administration alone were significantly lower both at 1 h (*P* < 0.05) and at 2 h (*P* < 0.03). The highest concentration of levowarfarin during the phenylbutazone regimen was achieved at 2 h in one subject, at 4 h in two subjects, at 8 h in two subjects, and at 12 h in one subject. The highest mean concentration of levowarfarin in plasma during the phenylbutazone regimen occurred at 8 h compared with 4 h for levowarfarin alone. Thus, the phenylbutazone regimen apparently resulted in a slower rate of absorption of levowarfarin from the pseudoracemate.

After the peak concentrations were reached, the plasma concentrations of levowarfarin declined by apparent first-order kinetics in all subjects for the duration of the experiments with and without phenylbutazone (Fig. 1). The mean concentrations of levowarfarin during the phenylbutazone regimen were significantly different from warfarin alone only at 168 h (*P* < 0.05). The AUC-warfarin for levowarfarin during the phenylbutazone regimen was increased in all six subjects, and the difference between the means with and without phenylbutazone was significant (*P* < 0.05) (Table I).

[^{12}C / ^{13}C]R,S(\pm)-warfarin. After administration of

the warfarin pseudoracemate alone, the highest concentration of racemic warfarin in plasma was achieved at 2 h in one subject, at 4 h in four subjects, and at 8 h in one subject. The highest mean concentration of racemic warfarin alone occurred at 4 h. When the warfarin pseudoracemate was administered during the phenylbutazone regimen, the highest concentration of racemic warfarin in plasma was achieved at 2 h in one subject, at 4 h in two subjects, at 8 h in two subjects, and at 12 h in one subject. The highest mean concentration of racemic warfarin during the phenylbutazone regimen occurred at 8 h, compared with 4 h for warfarin alone. The mean concentration of racemic warfarin during the phenylbutazone regimen compared with racemic warfarin alone was significantly lower both at 1 and 2 h (*P* < 0.05). Thus, the phenylbutazone regimen resulted in a slower rate of absorption of racemic warfarin from the pseudoracemate.

After the peak concentrations were reached, the plasma concentrations of racemic warfarin declined by apparent first-order kinetics in all subjects for the duration of the experiments both with and without phenylbutazone. The mean concentrations of racemic warfarin during the phenylbutazone regimen were significantly different from administration of warfarin alone only at 60 and 84 h (*P* < 0.02). The total AUC-warfarin for racemic warfarin during the phenyl-

butazone regimen was reduced for all subjects. The mean AUC-warfarin for racemic warfarin during the phenylbutazone regimen compared with that for racemic warfarin alone was reduced to a highly significant degree ($P < 0.01$) (Table I).

One-stage prothrombin activity. After administration of the single dose of the warfarin pseudoracemate alone, all six subjects had detectable hypoprothrombinemia after 12 h (Fig. 1). The most marked depression occurred 48–96 h after the warfarin dose, and the return to 100% of normal activity occurred 144–240 h after it. The mean of the lowest one-stage prothrombin activity occurred at 60 h. The magnitude of the AUC-prothrombin time for the one-stage prothrombin activity varied from 56 to 121 U; the mean \pm SEM for AUC-prothrombin time was 85 ± 10 U.

After administration of the warfarin pseudoracemate during the phenylbutazone regimen, all six subjects had detectable hypoprothrombinemia after 12 h. The most marked depression occurred 72–120 h after the warfarin dose, and the return to 100% of normal activity occurred at 240 h in three subjects and occurred after 240 h in the other three subjects. The mean of the lowest one-stage prothrombin activity occurred 84 h after the warfarin dose during the phenylbutazone regimen, compared with 60 h after the warfarin dose alone. The difference between the mean of the hypoprothrombinemia for warfarin alone and during the phenylbutazone regimen in the first 36 h after the warfarin dose was not significant, from 48 to 60 h was significant ($P < 0.02$), from 72 to 216 h was highly significant ($P < 0.01$), and was not significant at 240 h. The AUC-prothrombin time for the one-stage prothrombin activity increased markedly in all six subjects. The mean \pm SEM was 158 ± 13 U, compared with a mean AUC-prothrombin time for warfarin alone of 85 ± 10 U, a highly significant augmentation ($P < 0.001$) of the hypoprothrombinemia during the phenylbutazone regimen.

Pharmacokinetic analysis of plasma concentrations of dextrowarfarin. A pharmacokinetic analysis was undertaken on the plasma concentrations of [^{12}C]R(+)-warfarin, or dextrowarfarin, after single doses of warfarin pseudoracemate with and without the phenylbutazone regimen (Table I). The means of plasma concentration at time zero and of Vd for warfarin alone were not significantly different from those for warfarin during the phenylbutazone regimen. The mean Vd was 9.0 liters for the warfarin alone and 8.8 liters for warfarin during the phenylbutazone regimen, or 11.9 and 11.7% of the average body weight, respectively. The mean \pm SEM for the half-life of dextrowarfarin was 41.2 ± 1.7 h for warfarin alone and 26.4 ± 1.6 h for warfarin during the phenylbutazone regimen, a highly significant difference ($P < 0.001$).

The mean \pm SEM area under the curve for the plasma concentration of warfarin, AUC-warfarin, was 354 ± 20 mg/liter \times h for warfarin alone and 210 ± 19 mg/liter \times h for warfarin during the phenylbutazone regimen, a highly significant difference ($P < 0.01$). The mean plasma clearance of dextrowarfarin was 2.61 ± 0.15 ml/min for warfarin alone and 4.25 ± 0.32 ml/min for warfarin during the phenylbutazone regimen, a highly significant increase in dextrowarfarin clearance ($P < 0.01$).

Pharmacokinetic analysis of plasma concentrations of levowarfarin. A pharmacokinetic analysis was undertaken on the plasma concentrations [^{13}C]S(–)-warfarin, or levowarfarin, after single doses of the warfarin pseudoracemate with and without the phenylbutazone regimen (Table I). The mean Vd was 7.8 ± 0.6 liters for warfarin alone and 9.3 ± 0.4 liters for warfarin during the phenylbutazone regimen, a highly significant difference ($P < 0.01$), and were 10.3 and 12.3% of body wt, respectively. The mean SEM for half-life of levowarfarin was 30.5 ± 5.7 h for warfarin alone and 40.0 ± 3.8 h for warfarin during the phenylbutazone regimen, a significant difference ($P < 0.05$). The mean \pm SEM for AUC-warfarin was 277 ± 33 mg/liter \times h for warfarin alone and 327 ± 25 mg/liter \times h for levowarfarin during the phenylbutazone regimen, a significant difference ($P < 0.05$). The mean \pm SEM for plasma clearance was 3.52 ± 0.46 ml/min for warfarin alone and 2.86 ± 0.24 ml/min for warfarin during the phenylbutazone regimen, a significant decrease in levowarfarin clearance ($P < 0.05$).

Pharmacokinetic analysis of plasma concentrations of pseudoracemic warfarin. A pharmacokinetic analysis of pseudoracemic warfarin after single doses of the warfarin pseudoracemate was undertaken on the sum of the plasma concentrations of [^{12}C]R(+)-warfarin and [^{13}C]S(–)-warfarin for each subject at every time tested. The means for Vd were not significantly different for warfarin alone and for warfarin during the phenylbutazone regimen. The mean Vd was 8.8 liters for pseudoracemic warfarin alone and 9.2 liters for pseudoracemic warfarin during the phenylbutazone regimen, or 11.7% and 12.2% of body wt, respectively. The mean \pm SEM half-life was 36.2 ± 3.2 h for pseudoracemic warfarin alone and 33.9 ± 1.8 h for pseudoracemic warfarin during the phenylbutazone regimen, an insignificant difference. The mean \pm SEM for AUC-warfarin was 630 ± 34 mg/liter \times h for pseudoracemic warfarin alone and 528 ± 41 mg/liter \times h for pseudoracemic warfarin during the phenylbutazone regimen, a significant difference ($P < 0.01$). The mean \pm SEM for plasma clearance was 2.96 ± 0.26 ml/min for warfarin alone and 3.43 ± 0.27 ml/min for warfarin during the phenylbutazone regimen, a significant increase in pseudoracemic warfarin clearance ($P < 0.05$).

DISCUSSION

The authenticity of pseudoracemic warfarin in both its synthesis and preparation was validated by direct comparison with commercial racemic warfarin in all the research subjects. The total hypoprothrombinemic effect was evaluated by comparison of the mean AUC-prothrombin time for pseudoracemic warfarin and for racemic warfarin. The mean difference was insignificant. Similarly, there was no significant difference in the AUC of the blood concentrations achieved when pseudoracemic and racemic warfarin were compared. The hypoprothrombinemic effect of pseudoracemic warfarin with daily phenylbutazone administration was 186% of the control value, which is similar to the augmentation by phenylbutazone previously reported for racemic warfarin in normal subjects (1, 2). Thus, the artificial admixture of synthetic [^{13}C]levowarfarin and [^{12}C]dextrowarfarin as pseudoracemic warfarin behaved pharmacologically like synthetic racemic warfarin.

Although the interaction of warfarin and phenylbutazone has been previously studied with racemic warfarin (1, 2) and with the separated enantiomorphs of warfarin (2), no previous data have been published on the fate of each enantiomorph in racemic warfarin during the interaction. This approach is important because of the widely divergent effects of phenylbutazone on the enantiomorphs of warfarin. Lewis and Trager (2) found in two subjects that phenylbutazone speeded up the metabolic disposition of dextrowarfarin and slowed down the metabolic disposition of levowarfarin in plasma. They studied the urinary metabolic products of racemic warfarin with and without phenylbutazone in two other subjects and concluded that the effect of phenylbutazone on the warfarin enantiomorphs administered together was similar to that on the separated enantiomorphs (2). Our study has confirmed that report by direct measurement of the warfarin enantiomorphs during their simultaneous administration as a pseudoracemate. The synthesis of one enantiomorph of a racemate with ^{13}C to prepare a [$^{12}\text{C}/^{13}\text{C}$]pseudoracemate may have wide application to other racemic drugs.

Phenylbutazone seemed to slow the gastrointestinal absorption rate of both dextrowarfarin and levowarfarin, as well as pseudoracemic warfarin. This effect of phenylbutazone on the absorption rate of warfarin hitherto has not been reported. These findings probably were not observed before because previous investigations of this drug interaction did not include early blood sampling. Racemic warfarin is completely absorbed in normal subjects, whereas the oral anticoagulant dicumarol is not (8). The absorption of racemic warfarin was not affected even by diseases

associated with severe gastrointestinal malabsorption. Cholestyramine in man interfered with the absorption of racemic warfarin causing it to appear in the stool (9), and also interfered with the absorption and subsequent hypoprothrombinemic effect of racemic phenprocoumon (10). Barbiturates did not affect the absorption of racemic warfarin, but did reduce the absorption of dicumarol (11). Antacid therapy had no effect on the absorption of racemic warfarin (12). The simultaneous ingestion of food interfered with the rate but not the extent of absorption of racemic warfarin in man (13), and actually enhanced the absorption of poorly absorbable dicumarol in one study in man (14). Thus, the impact of phenylbutazone on the absorption of pseudoracemic warfarin is similar to that of food: reduction in the rate, but not the extent of racemic warfarin absorption, and no effect on its bioavailability or hypoprothrombinemic effect.

Racemic warfarin acid is the rodenticidal form of the drug, whereas racemic warfarin sodium (Coumadin; Panwarfin, Abbott Laboratories, North Chicago, Ill.) or racemic warfarin potassium (Athrombin-K, The Purdue Frederick Company, Norwalk, Conn.) is the form used clinically. All the [^{12}C]- or [^{13}C]warfarin preparations used in this study were warfarin acids. Perhaps, the effect of phenylbutazone on the absorption rate of pseudoracemic warfarin acid, dextrowarfarin acid, and levowarfarin acid would not have occurred with the form used clinically, racemic warfarin sodium. However, this possibility is unlikely both because of conversion to warfarin acid on contact with the acid content of the stomach, and because previous studies in man comparing tablets of racemic warfarin acid to racemic warfarin sodium showed no significant differences in the gastrointestinal absorption rate of the drug, bioavailability, time of peak blood levels, AUC-warfarin, AUC-prothrombin time, Vd, or half-life (8, 15).

The following mechanism can be proposed for the interaction of phenylbutazone with racemic warfarin. Dextrowarfarin undergoes metabolic transformation primarily by reduction of the ketone function of the acetyl side chain to two or more secondary alcohols, which are excreted mainly by the kidney into the urine (16). Levowarfarin undergoes metabolic transformation primarily by oxidation via ring hydroxylation of the coumarin nucleus to 7-hydroxylevowarfarin, which is excreted by the liver mainly into the bile and eventually into the stool (17). It is proposed that the activity of the enzymes controlling the ring oxidation of levowarfarin may be inhibited by phenylbutazone. Studies with single enantiomorphs of warfarin support this suggestion (2). This inhibition would impair the total body clearance of levowarfarin and lead to higher blood levels and a longer half-life

in plasma for unchanged levowarfarin. It is also proposed that the activity of the enzymes controlling the side-chain reduction of dextrowarfarin perhaps is increased via enzymatic induction by phenylbutazone. This enzymatic stimulation would increase the total body clearance of dextrowarfarin and lead to lower blood levels and a shorter half-life in plasma for unchanged dextrowarfarin.

The greater quantity in the body of the more potent anticoagulant levowarfarin, even with a lesser quantity of total warfarin, would hinder the synthesis of the vitamin K-dependent clotting factors more completely and for a longer time in the presence of phenylbutazone (18). This lessened synthesis of clotting factors would lead to a greater hypoprothrombinemic effect with both racemic and pseudoracemic warfarin. However, the alterations in the total body clearances of the enantiomorphs of warfarin by phenylbutazone, particularly that of levowarfarin, are modest compared with the marked augmentation of the hypoprothrombinemia in every subject tested. These data suggest that other factors contribute to the interaction.

A phenylbutazone-induced increase in the free fraction of racemic warfarin was reported by Aggeler and co-workers (1) and confirmed by Lewis et al. (2). The importance of this alteration of albumin-bound warfarin by phenylbutazone was observed by Tillement et al. (19). They found that phenylbutazone increased the free fraction of the three coumarin anticoagulants tested (acenocoumarol, ethyl biscoumatate, and warfarin) but had no effect on that of the indanedione anticoagulants tested (fluorophenindione and phenindione). They correlated these binding data with the marked hypoprothrombinemic augmentation of coumarin anticoagulants and the absence of this interaction with indanediones (19). Our data showed a highly significant increase in the Vd of levowarfarin, which is consistent with an increase in the free fraction of levowarfarin. The half-life of oral pseudoracemic warfarin administration by the one-compartment analysis reported herein, 36 h, is similar to administration of truly racemic warfarin intravenously by a two-compartment open model, 35 h (20). It is also possible that the interaction results from a direct effect of phenylbutazone on the synthesis or the degradation of the vitamin K-dependent clotting factors. This possibility seems unlikely because phenylbutazone has no hypoprothrombinemic action by itself and has no effect on the degradation rate of clotting factors as evidenced by comparable prolongation of the prothrombin time for the first 48 h after the warfarin dose with and without phenylbutazone (21). Thus, the most likely explanation for the interaction is a complex combina-

tion of effects: protein displacement and stereoselective metabolic changes.

The study reported herein demonstrated a stereoselective interaction of phenylbutazone on pseudoracemic warfarin. Other studies using separated enantiomorphs of racemic warfarin also demonstrated a stereoselective interaction with phenylbutazone: decreased clearance of levowarfarin and increased clearance of dextrowarfarin (3). Phenylbutazone augmented the hypoprothrombinemia of levowarfarin but had little or no effect on that of dextrowarfarin. These findings suggest that the interaction of racemic warfarin and phenylbutazone may be lessened if racemic warfarin were replaced by dextrowarfarin for long-term therapy. Thus, long-term therapy with oral anticoagulants could become more stable and less dangerous. Substantiation of this possibility requires further detailed investigations to clarify the toxicity of dextrowarfarin at higher dosage and any significant interaction with phenylbutazone at those doses.

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