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PHENYLBUTAZONE–WARFARIN INTERACTION IN MAN: FURTHER STEREOCHEMICAL AND METABOLIC CONSIDERATIONS

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1 The pharmacokinetics and urinary metabolic profile of R and S-warfarin, following administration of a 1.5 mg/kg oral dose of racemic warfarin, alone and 4 days into an oral regimen of 100 mg phenylbutazone three times a day, was investigated in three volunteers using a stereospecific h.p.l.c. fluorescent assay.

2 The mean elimination half-life of S-warfarin was increased from 25 to 46 h during phenylbutazone administration, whilst that of the R-isomer was decreased from 37 to 25 h.

3 The peak unbound concentrations of both warfarin enantiomers were higher during phenylbutazone administration, due to displacement. Displacement was not stereoselective.

4 The unbound clearance of more potent S-warfarin is decreased by four-fold during phenylbutazone administration, due to substantial inhibition of both 6- and 7-hydroxylation, significant pathways of elimination of S-warfarin in the absence of phenylbutazone.

5 The unbound clearance of R-warfarin is almost unchanged during phenylbutazone administration, due to the marginal effect of phenylbutazone on 6- and 7-hydroxylation, themselves minor pathways of elimination of this enantiomer in the absence of phenylbutazone.

6 The stereoselective reduction of S- and R-warfarin, to their respective SS and RS-alcohols, is also substantially inhibited during phenylbutazone administration.

7 Collectively the data point to the complex effect of phenylbutazone administration on warfarin's pharmacokinetics.

Keywords phenylbutazone warfarin interaction stereochemistry

Introduction

Perhaps the most widely publicised and documented interaction of the oral anticoagulants is between the anti-inflammatory drug phenylbutazone and warfarin; when concomitantly administered with warfarin, phenylbutazone causes a profound potentiation of the hypoprothrombinaemic response, which can be life-threatening (Aggeler *et al.*, 1967). As both warfarin and phenylbutazone bind strongly to the same site on albumin, the explanation commonly offered for the interaction is that phenylbutazone displaces warfarin from albumin thereby increasing the unbound (and pharmacologically active) concentration of warfarin (Anon, 1976). Whilst displacement is observed, the case for this hypothesis explaining the potentiation of warfarin is weakened on two accounts. Firstly, theory predicts that potentiation due to displacement should be transient (Aarons & Rowland, 1981), yet the interaction persists as long as phenylbutazone is co-administered (Sellers & Koch-Weser, 1970). And secondly, many other drugs besides phenylbutazone displace warfarin from albumin *in vivo* yet they do not potentiate warfarin's effect under clinical use.

Further insight into the interaction came through stereochemical considerations. Warfarin is administered as a racemic mixture, i.e. an equal mixture of two optical isomers. In man, S(−)-warfarin is five times more potent (Eble *et al.*, 1966), and is eliminated more rapidly (Hewick & McEwan, 1973) than the R(+)-isomer. When co-administered with phenylbutazone, the elimination half-life of S(−)-warfarin is increased (and clearance reduced), while

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the elimination half-life of the R(+)-warfarin is reduced (and clearance increased). This reduced elimination of the S(−)-warfarin explains, at least in part, the potentiating effect of phenylbutazone. Thus, phenylbutazone has been described as acting stereoselectively, inhibiting the metabolism of the S-isomer but inducing the metabolism of the R-isomer (Lewis *et al.*, 1974; O'Reilly *et al.*, 1980). These interpretations were based on measurements in plasma, following either the independent administration of warfarin isomers, or the administration of a pseudoracemate (C^{12}(R), C^{13}(S)) and determining the isomers by mass spectrometry. Measurement of the more important unbound concentration of each isomer in plasma and of the enantiomeric metabolites of warfarin has so far been lacking.

We report our findings of the phenylbutazone-warfarin in man using a stereospecific assay for the analysis of the enantiomers of warfarin and of its known metabolites, together with measurement of the unbound enantiomeric warfarin concentrations.

Methods

The study has been described previously (O'Reilly *et al.*, 1980), and involved oral administration of 1.5 mg/kg of racemic warfarin to volunteers before and 4 days into a 12-day oral regimen of 100 mg phenylbutazone three times a day, with a 4-week rest period between the two phases. The study was approved by the local ethical committee and the results reported below were obtained in three healthy volunteers who gave their informed consent.

Serial blood samples and complete serial urine samples were obtained over a 10-day study period following each dose of warfarin, with a control sample of blood and urine being taken just prior to each phase of the study. The samples had been kept at −20° until analysed.

The stereochemical assay involves coupling the enantiomers with the optically active reagent, carbobenzyloxy-L-proline, and separating the diastereoisomeric pairs by h.p.l.c., with fluorescence detection after post-column aminolysis of the diastereoisomeric esters (Banfield & Rowland, 1983). The determination limit for any enantiomer is in the order of 50–100 ng. No interfering peaks were observed when either control plasma and urine samples, or control plasma and urine spiked with phenylbutazone, and its metabolites, oxyphenbutazone and γ-hydroxyphenylbutazone, were taken through the assay procedure.

The fraction unbound (*fu*) of each warfarin enantiomer was determined, using a 1 ml plasma sample, by equilibrium dialysis (4 h) at 37°C, against a pH 7.4 phosphate buffer, after addition of 15 μg racemic warfarin to the plasma just prior to dialysis.

The terminal half-life ($t_{½}$) was determined from the slope of the regression of log concentration against time. The area under the plasma warfarin enantiomer concentration-time curve (AUC) was determined by trapezoid approximation up to the last observation, with extrapolation beyond by dividing the last observation by the terminal rate constant ($0.693/t_{½}$). The extrapolated area did not exceed 4% of the calculated total AUC value. Total plasma clearance (CL) of each enantiomer was obtained by dividing the administered dose (one half the racemic dose) by the respective AUC value. The volume of distribution (V), was calculated from the formula (Rowland & Tozer, 1980)

$$V = 1.44.\mathrm{CL}.t_{½} \quad (1)$$

The fraction of warfarin enantiomer unbound (*fu*), during each phase of the study, was taken as the mean of at least five determinations of *fu*, each measurement involving use of plasma sample taken at a different times during the respective study. The unbound concentration of each warfarin enantiomer was calculated by multiplying the total plasma concentration by the respective mean value of *fu*. The corresponding unbound clearance (CLu) and unbound volume of distribution (Vu) were calculated from the formulae (Rowland & Tozer, 1980)

$$\mathrm{CLu} = \mathrm{CL}/fu \quad (2)$$

$$V\mathrm{u} = V/fu \quad (3)$$

Results

The stereospecific h.p.l.c. fluorescent assay permits determination of the enantiomers of warfarin and of its known metabolites (6- and 7-hydroxywarfarin, and RS and SS-warfarin alcohols). There is no interference in the assay by phenylbutazone or its metabolites, oxyphenbutazone and γ-hydroxyphenylbutazone (Banfield & Rowland, 1983).

Figure 1 shows the total and estimated unbound concentrations of S- and R-warfarin in one subject, before and during administration of phenylbutazone. Similar results were obtained in the other two subjects. Although the peak total plasma concentration of each enantiomer is not changed during phenylbutazone administration the respective peak unbound concentration is increased.

Table 1 summarises the results for the three subjects studied. The mean half-life of S-warfarin increased from 25 h (control) to 46 h during phenylbutazone administration, whilst that of R-warfarin decreased from 37 h (control) to 25 h. The total plasma clearance of S-warfarin decreased from 4 ml h^{-1} kg^{-1} to 2.2. ml h^{-1} kg^{-1} during phenylbutazone

Table 1 Summary of pharmacokinetic data

Subject	*1*		*2*		*3*		*Average*	
	Control	*During phenylbutazone*	*Control*	*During phenylbutazone*	*Control*	*During phenylbutazone*	*Control*	*During phenylbutazone*
Half-life (h)								
S-Warfarin	22	47	33	46	19	42	25	46
R-Warfarin	33	24	43	25	34	26	37	25
Clearance (ml h^{-1} kg^{-1})								
S-Warfarin	4.4	2.1	3.2	2.3	4.5	2.1	4.0	2.2
R-Warfarin	2.9	4.0	2.4	4.2	2.6	3.3	2.6	3.8
Volume of distribution (*l* kg^{-1})								
S-Warfarin	0.14	0.14	0.15	0.15	0.13	0.13	0.14	0.14
R-Warfarin	0.14	0.15	0.15	0.15	0.13	0.13	0.14	0.14
% Unbound								
S-Warfarin	0.44	1.1	0.41	1.1	0.44	0.87	0.43	1.0
R-Warfarin	0.49	1.1	0.60	1.1	0.60	0.93	0.56	1.0
Unbound clearance (ml h^{-1} kg^{-1})								
S-Warfarin	1000	190	780	210	1020	230	940	210
R-Warfarin	600	360	400	380	430	350	480	360
Unbound volume of distribution (l kg^{-1})								
S-Warfarin	32	13	37	14	29	14	33	14
R-Warfarin	29	13	25	14	21	13	25	13

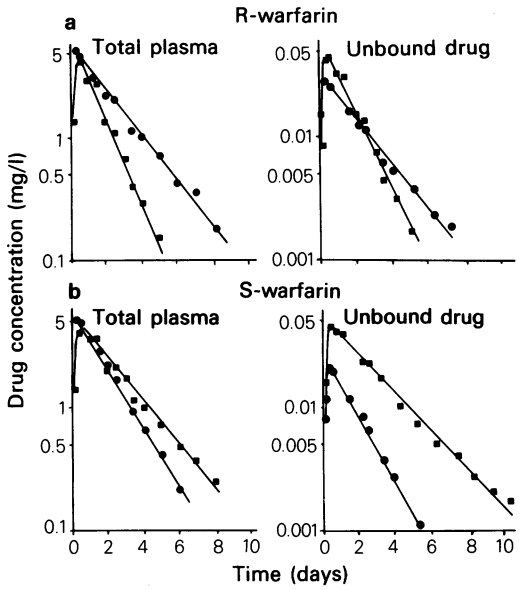

Figure 1 The total and estimated unbound concentrations of R(+)-warfarin (a) and S(−)-warfarin (b), following oral administration of 1.5 mg/kg racemic warfarin to a subject before (●), and 4 days into a regimen of 100 mg phenylbutazone three times a day (■).

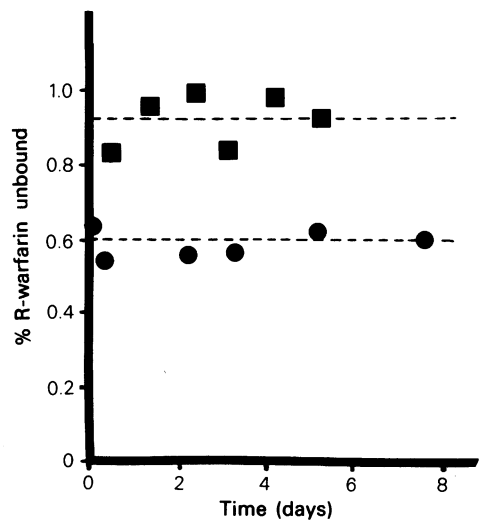

Figure 2 Lack of a systemic change in the fraction of R-warfarin unbound in plasma with time during the control phase (●) and during phenylbutazone administration (■). The dotted horizontal lines denote the mean values during the respective phases.

administration, whilst that of R-warfarin increased from 2.6 to 3.8 ml h^{-1} kg^{-1}. The mean volume of distribution based on total plasma drug concentration, was unchanged (0.14 l kg^{-1}) for both enantiomers during phenylbutazone administration.

Displacement of warfarin by phenylbutazone is seen. In the three subjects, the mean fraction of S-warfarin unbound increased from 0.43 to 1.03% between control and phenylbutazone administration, while that of R-warfarin increased from 0.56 to 1.03%. The fraction unbound of R and S-warfarin, control and during phenylbutazone administration, showed no systematic change with time (e.g. Figure 2). The unbound clearance of S-warfarin decreased from 940 to 210 ml h^{-1} kg^{-1} during phenylbutazone administration, whilst that of R-warfarin decreased marginally from 480 to 360 ml h^{-1} kg^{-1}. The unbound volume of distribution decreased for both enantiomers following phenylbutazone administration, from 33 to 14 l kg^{-1} for S-warfarin and from 25 to 13 l kg^{-1} for R-warfarin.

Table 2 shows the 10-day mean urinary excretion data of warfarin metabolites when given alone and during phenylbutazone administration. For S-warfarin the excretion of both 6- and 7-S-hydroxywarfarin is substantially decreased (by 55 and 70% respectively) whilst that of SS-warfarin alcohol is marginally increased (by 11%). For R-warfarin the excretion of all three metabolites, 6- and 7-R-hydroxywarfarin and RS-warfarin alcohol is decreased (by 47.70 and 82% respectively) during phenylbutazone administration. Only traces of R and S-warfarin were detected in urine. Together, in the case of S-warfarin, the metabolites account for 58% of the dose administered in the control period and 22% during phenylbutazone, the corresponding values for R-warfarin are 29.5 and 11.7%, respectively.

Discussion

The clinical phase of the study was performed several years ago (O'Reilly *et al.*, 1980). Nonetheless, the plasma concentrations of both enantiomers of warfarin determined by the presently-employed h.p.l.c. method were not significantly different from those determined by a mass-spectroscopic method, used at the time of the study (Banfield & Rowland, 1983), suggesting that warfarin is stable at −20°C for at least several years. Warfarin metabolic data were not previously determined, but observations in our laboratory suggest that aqueous solutions kept at −20°C are stable for at least many months.

Although sensitive, the h.p.l.c. method could not determine the unbound concentration of each warfarin enantiomer in all plasma samples, warfarin being very highly bound in plasma to albumin. *In*

Table 2 10-day mean[a] urinary excretion of warfarin metabolites

	Cumulative amount excreted (mg)[b]		
	Control	*During phenylbutazone*	*% change*
S(−)-warfarin			
6-S-hydroxywarfarin	6.70	3.0	−55
7-S-hydroxywarfarin	21.0	6.3	−70
SS-warfarin alcohol	2.80	3.1	+11
Total (%) dose	30.5 (58.0)	12.4 (22.0)	
R(+)-warfarin			
6-R-hydroxywarfarin	8.93	4.75	−47
7-R-hydroxywarfarin	1.78	0.53	−70
RS-warfarin alcohol	4.80	0.86	−82
Total (% dose)	15.5 (29.5)	6.14 (11.7)	

[a] Mean of data in three volunteers
[b] Expressed in warfarin equivalents

vitro studies by us have shown that the fraction of warfarin unbound in plasma is independent of concentration up to 25 μg racemic warfarin/ml, and so 15 μg racemic warfarin was added to each plasma sample subjected to equilibrium dialysis to facilitate determination of the fraction unbound. Limited sample size prevented binding measurements being done on all plasma samples. Nonetheless, as the fraction unbound of R and S-warfarin, control and during phenylbutazone administration, did not change with time during a study it was assumed that the mean fraction unbound determined for each phase was applicable to all the respective plasma samples.

The higher initial unbound concentration of both warfarin isomers during phenylbutazone administration is due to displacement of them by phenylbutazone from albumin, to which it is highly bound (in both plasma and the remaining extracellular fluid). This displacement effect is reflected in the smaller unbound volume of distribution of both enantiomers during phenylbutazone administration. The data suggests no strong evidence of stereoselective displacement of warfarin by phenylbutazone.

For drugs like warfarin, well absorbed (Breckenridge & Orme, 1973), of low clearance (and extraction) and predominantly metabolised, the area under the unbound concentration-time curve, following oral administration, is a measure of the intrinsic clearance (CLu) of the metabolic enzymes responsible for drug elimination (Wilkinson & Shand, 1975). The (approximately four-fold) greater unbound area for the S(−)-isomer during phenylbutazone administration indicates that the intrinsic clearance of this isomer is reduced, supporting the earlier suggestion of metabolic inhibition by phenylbutazone (Lewis *et al.*, 1974). In contrast, measurement shows that the area under the unbound concentration of the R(+)-isomer is decreased by only 25% during phenylbutazone administration, indicating little if any change in the intrinsic clearance of this isomer. The shorter half-life of R(+)-warfarin in the presence of phenylbutazone is, therefore, not due to induction of metabolic enzymes but rather is in keeping with the prediction of predominantly simple displacement from albumin, of such a drug of low clearance and small volume of distribution (Nagashima & Levy, 1969; Øie *et al.*, 1980). It does appear therefore that phenylbutazone interacts stereoselectively with warfarin.

The urinary metabolic data, however, suggest a more complicated situation. As shown in Table 2, for both enantiomers the excretion (an index of formation) of 6- and 7-hydroxywarfarin is reduced during phenylbutazone administration. So is that of RS-warfarin alcohol, while the excretion of SS-warfarin alcohol is slightly increased.

Unbound clearance associated with the formation of a particular metabolic pathway, CLu_m is a better index of metabolic activity than is cumulative excretion of the metabolite. The latter tends to reflect more the relative importance of the affected pathway to overall elimination of the drug. Thus, if all the drug is converted to a single metabolite then the total amount of the metabolite formed, and excreted, will be the same even if the pathway is inhibited. The value of CLu_m for each metabolite is given by the relationship

$$CLu_m = \frac{Ae\,(m, \infty)}{fe(m).(AUC)_u} \qquad (4)$$

where Ae (m, ∞) is the cumulative amount of metabolite excreted unchanged, $fe(m)$ is the fraction of the formed metabolite that is excreted unchanged, and $(AUC)_u$ is the total area under the unbound drug

concentration-time curve. In man, conjugates of 6 and 7-hydroxywarfarin have not been found in urine, and when administered separately RS- and SS-alcohol are excreted to a large extent unchanged (Lewis & Trager, 1970). Some of the metabolites of warfarin appear in faeces (Toon & Trager, personal communication) presumably via biliary excretion, as warfarin is completely absorbed. Nonetheless, while exact values are lacking, it seems reasonable to conclude that the values of *fe*(m) for each of the warfarin metabolites measured is high. In the subsequent analysis *fe*(m) is assumed to be one, and that this value is not affected by the presence of phenylbutazone. As such the calculated values of CLu_m represent the minimum estimates.

In Table 3 are listed the mean calculated values of CLu_m, and of the fraction of enantiomer eliminated by that pathway, *fm*, (given by CLu_m/CLu), for each metabolite, before and during phenylbutazone administration. A pattern is seen to emerge. Formation of all measured metabolites of both warfarin enantiomers is reduced during phenylbutazone administration. However, the overall unbound clearance of R-warfarin is only marginally diminished (from 480 to 360 ml h^{-1} kg^{-1}) because, even together, these affected pathways account for relatively little (24%) of the dose of this enantiomer eliminated, when administered alone.

7-hydroxylation is a far more important pathway of elimination of S-warfarin (*fm* = 46%). Moreover, this and indeed all pathways of S(−)-warfarin metabolism are depressed to a much greater extent (73–93%) than the corresponding metabolic pathways for R-warfarin (58–86%) during phenylbutazone treatment. These observations explain in part, but not totally, the decrease in the unbound clearance of S-warfarin, from the control value of 940 to 210 ml h^{-1} kg^{-1} during phenylbutazone administration. Together the formation of all measured metabolic pathways of S-warfarin are depressed by 430 ml h^{-1} kg^{-1} (from 480 to 45 ml h^{-1} kg^{-1}, Table 3). It would appear that either other pathways, not measured, are also depressed during phenylbutazone administration, or that biliary excretion of metabolites is significant.

Stereoselective inhibition by phenylbutazone of warfarin hydroxylation is seen. Formation of both 6- and 7-R-hydroxywarfarin is moderately reduced, to approximately 34% of control values, whereas formation of both 6- and 7-S-hydroxywarfarin is substantially reduced to just 8.5% of the corresponding control values (Table 3). One possible explanation, for the similar degree of depression of 6- and 7-hydroxylation of each warfarin enantiomer, is formation of these metabolites via a common 6-7 arene oxide, with phenylbutazone inhibiting formation of the two enantiomeric arene oxides stereoselectively. Studies in rats indicate that several hepatic enzymes can hydroxylate warfarin, some via arene oxide formation, although formation of the 6-7 arene oxide has not been specifically demonstrated (Pohl *et al.*, 1976, 1977; Fasco *et al.*, 1978). Alternatively, 6- and 7-hydroxylation could occur by an insertion mechanism, perhaps involving different forms of cytochrome-P450, which phenylbutazone inhibits stereoselectively.

To what extent the above observations explain other findings is conjectural at this stage. It is interesting to note, however, that both the trimethoprin-sulphamethoxazole combination and metronidazole, which potentiate the anticoagulant effect of warfarin, diminish the elimination of S(−)-warfarin, without affecting the kinetics of the R(+)-isomer (O'Reilly, 1976; O'Reilly & Motley, 1979). Enantiomeric metabolic data would clarify the interpretation.

Many drugs have one or more chiral centres and are administered therapeutically as a racemic mixture. With the increasing development of stereospecific assays (Silber & Riegelman, 1980; Eichelbaum *et al.*, 1981), differences in pharmacokinetic behaviour between enantiomers is becoming apparent and the detection of 'stereospecific' drug interactions is likely. The present findings with the classic phenylbutazone–warfarin interaction stress the need to measure stereochemical metabolic patterns before reaching conclusions as to the nature of the mechanism of any interaction.

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Table 3 Estimated unbound formation clearance of metabolites

	Unbound formation clearance (ml h^{-1} kg^{-1})			fm (CLu_m/CLu)	
	Control	*During phenylbutazone*	*% change*	*Control*	*During phenylbutazone*
S(−)-warfarin					
6-S-hydroxywarfarin	106	10.9	−90	0.113	0.052
7-S-hydroxywarfarin	330	22.7	−93	0.351	0.108
SS-warfarin alcohol	42	11.2	−73	0.045	0.053
R(+)-warfarin					
6-R-hydroxywarfarin	68.5	28.9	−58	0.14	0.080
7-R-hydroxywarfarin	12.9	3.3	−74	0.027	0.009
RS-warfarin alcohol	34	5.3	−86	0.071	0.051

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