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Stereoselective interaction between the R enantiomer of warfarin and cimetidine

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1 The stereoselectivity of the pharmacokinetic interaction between warfarin and cimetidine was investigated in eight healthy volunteers.

2 The warfarin enantiomers were given separately as single doses (15 mg) alone and during chronic administration of cimetidine (1 g day^{-1}).

3 Cimetidine did not interact with S warfarin but there was an interaction with the R enantiomer of warfarin. Cimetidine caused a significant increase in the mean plasma half-life of R warfarin (from 47.8 h to 57.8 h) and a significant decrease in its mean plasma clearance (from 2.3 to 1.7 ml h^{-1} kg^{-1}) ($P < 0.02$).

4 Administration of a pharmacological dose of vitamin K_1 together with the enantiomers of warfarin was necessary clinically and resulted in elevation of vitamin K_1 2,3-epoxide concentrations, which were similar in each case.

Keywords warfarin cimetidine interaction stereoselectivity

Introduction

Cimetidine interacts with warfarin resulting in increased plasma concentrations of the latter and a prolonged prothrombin time (Serlin *et al.*, 1979). The basis of this interaction has not been established but is thought to involve inhibition of drug metabolism, as substituted imidazoles such as cimetidine are potent inhibitors of drug oxidation (Somogyi & Gugler, 1982).

Warfarin is administered as a racemic mixture of two optically active isomers, R (+) and S (−) warfarin. There is considerable inter-individual variation in the relative potency of the two enantiomers. The mean potency of the S enantiomer has been reported as 3.8 (Breckenridge *et al.*, 1974), 3.4 (O'Reilly, 1974) and 2.7 (Wingard *et al.*, 1978) times that of the R enantiomer; however, in all the volunteers studied the S enantiomer was more potent. Several drug interactions with warfarin have been shown to have a stereoselective basis: metronidazole (O'Reilly, 1976); cotrimoxazole (O'Reilly, 1980); phenylbutazone (Lewis *et al.*, 1974); and sulphinpyrazone (O'Reilly, 1982). In each case the inhibition of metabolism has been predominantly that of the S enantiomer. We wished to see if the interaction with cimetidine was also stereoselective.

In addition, we took the opportunity to investigate any stereoselectivity in the mechanism of action of warfarin. This was done by the measurement of plasma vitamin K_1 2,3-epoxide concentrations, after the administration of a pharmacological dose of vitamin K_1. Coumarin anticoagulants are thought to inhibit the reduction of vitamin K_1 2,3-epoxide.

Methods

Plan of study

Eight volunteers entered the study having given informed consent. Approval was obtained from the Mersey Regional Ethics Committee.

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Volunteers took an oral dose (15 mg) of a single enantiomer of warfarin, each volunteer receiving both enantiomers with and without cimetidine. The order of the four parts of the study was randomised for each volunteer. Cimetidine, given in the dose of 200 mg three-times daily and 400 mg at night, was started 4 days before the administration of the warfarin and continued until the collection of the last blood sample.

The warfarin enantiomers (a gift from Ward Blenkinsop, 97% pure) were prepared by dissolving the solid in 0.5 M NaOH, adjusting the pH to 8.5 with 0.5 M HCl, and adjusting the final volume to 10 ml per dose. Vitamin K_1 (20 mg Konakion®), diluted in 20 ml 0.9% saline, was given intravenously over 20 min, together with the warfarin to prevent prolongation of the prothrombin time. Any prolongation of the prothrombin time (> 15 s) was corrected with oral vitamin K_1 (10 mg).

Blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h.

Analytical methods

Plasma warfarin enantiomer concentrations were determined using a normal phase high performance liquid chromatography assay (Breckenridge *et al.*, 1985).

Vitamin K_1 (the biologically active *trans*-isomer) and vitamin K_1 2,3-epoxide plasma concentrations were determined using a normal phase high performance liquid chromatography assay (Wilson & Park, 1983).

Prothrombin times were determined by the one stage technique using Manchester Comparative Thromboplastin and an automated coagulometer.

An *in vitro* experiment was carried out to determine whether cimetidine could displace warfarin from albumin. Non-radiolabelled racemic warfarin (500 ng ml^{-1}) was added to phosphate buffer (pH 7.4, 0.1 M) containing cimetidine at concentrations ranging from 0.1 to 100 μg ml^{-1}. Tracer amounts (< 0.1 μCi) of racemic [^{14}C]-warfarin (Radiochemical Centre, Amersham, specific activity 55 mCi $mmol^{-1}$) were added and the warfarin-cimetidine solution was dialysed at 37 °C against fresh human plasma for 16 h using a Dianorm apparatus (MSE).

Calculations

The vitamin K_1 plasma concentration *vs* time data were analysed according to a two-compartment model using an non-linear regression programme as described previously (Park *et al.*, 1984), to obtain the terminal half-life ($t_{½}$) and elimination rate constant (λ_z). The area under the plasma concentration-time curves (AUC) for warfarin, vitamin K_1 and vitamin K_1 2,3-epoxide were determined by trapezoidal approximation up to the last observation, with extrapolation beyond by dividing the final concentration by λ_z. Total plasma clearance (CL) of each warfarin enantiomer was obtained by dividing the administered dose by the respective AUC value, assuming complete absorption. The volume of distribution (V) was calculated from

$$V = \frac{CL}{\lambda_z}$$

The area extrapolated was under 10% in all cases, with the single exception of volunteer PW where the extrapolated area was 12.2% for S warfarin in the presence of cimetidine.

The concentration of free warfarin in plasma samples was determined using the method described by Giacomini *et al.* (1984), which takes into account the effect of volume shift during dialysis.

$$\text{Fraction unbound} = \frac{C'_B}{C^\circ_{bnd} + C'_B}$$

where C'_B is the concentration of free warfarin in the buffer after dialysis and C°_{bnd} is the concentration of bound drug had no volume shift occurred. The value of C°_{bnd} is obtained from

$$C^\circ_{bnd} = C'_{bnd}\,(1 + \delta)$$

where C'_{bnd} represents the bound drug concentration obtained after dialysis C'_{bnd} is obtained from

$$C'_{bnd} = C'_P - C'_B$$

where C'_P is the concentration of warfarin in the plasma after dialysis. δ is the fractional increase in the volume of the plasma due to the osmotic water shift and can be approximated as follows:

$$\delta = \frac{P}{P'} - 1$$

where P and P′ are the protein concentrations in the plasma before and after dialysis. In this experiment the value of δ was 0.13.

Statistical analysis was performed using Student's t-test for paired data.

Results

Adverse drug reactions

Three of the eight volunteers experienced adverse drug reactions during the trial. DR who

had pre-existing Wolff-Parkinson-White syndrome suffered a bradycardia 96 h after starting the cimetidine, and had to drop out of the study. IB experienced hypotension, facial flushing and sweating during the infusion of vitamin K_1, despite the fact that the rate of infusion was considerably less than 1 mg min^{-1}. He remained in the study but received oral vitamin K_1 thereafter. MO developed a severe generalised erythematous maculo-papular rash on receiving cimetidine on the second occasion. He discontinued his cimetidine 124 h after taking the R warfarin, but as there was no subsequent change in the elimination of the warfarin, his data have been included. For DR only the vitamin K data are included whereas for IB there are no vitamin K data. One volunteer (JH) had to drop out of the study for personal reasons, and data for R warfarin only are included.

Pharmacokinetics

The individual and mean data for the pharmacokinetics of both enantiomers of warfarin are shown in Table 1. There was a significant increase in both the AUC ($P < 0.01$) and the half-life ($P < 0.002$) of R warfarin following cimetidine and also a significant decrease in plasma clearance ($P < 0.02$). There was no consistent change in the pharmacokinetic parameters of S warfarin or the volume of distribution for either enantiomer following treatment with cimetidine. The results for a single volunteer (BH), which are typical, are shown in Figures 1 and 2.

The individual and mean data for the pharmacokinetics of vitamin K_1 and the 2,3-epoxide are shown in Table 2 and Figure 3. There was no difference in the formation of vitamin K_1 2,3-epoxide in the presence of R or S warfarin. Vitamin K_1 and vitamin K_1 2,3-epoxide plasma concentrations were below the limit of assay sensitivity (25 ng ml^{-1}) both prior to and 72 h after the administration of the intravenous vitamin K_1. Oral vitamin K_1 to correct prolongation of the prothrombin time was not required until 72 h after the intravenous vitamin K_1. Cimetidine (0.1 to 100 μg ml^{-1}) had no effect on the plasma protein binding of racemic warfarin *in vitro* (Table 3).

Discussion

The pharmacodynamic interaction between warfarin and cimetidine has been previously documented (Serlin *et al.*, 1979). The original study suggested that the nature of the interaction was due to an alteration of the pharmacokinetics of warfarin by cimetidine. The aim of this study was to determine whether this pharmacokinetic interaction is stereoselective.

Table 1 Pharmacokinetics of the enantiomers of warfarin

		R				*S*			
Volunteer		*AUC* (μg ml^{-1} h)	$t_{1/2}$ (h)	*CL* (ml h^{-1} kg^{-1})	V (l kg^{-1})	*AUC* (μg ml^{-1} h)	$t_{1/2}$ (h)	*CL* (ml h^{-1} kg^{-1})	V (l kg^{-1})
NK	W	99.5	44.4	2.04	0.13	76.0	44.6	2.67	0.17
	W + C	123.3	56.8	1.64	0.13	64.3	34.6	3.15	0.16
MO	W	89.2	47.3	2.37	0.16	43.9	23.2	4.81	0.16
	W + C	126.9	58.7	1.67	0.14	59.0	30.7	3.58	0.16
BH	W	97.7	42.1	2.05	0.12	52.4	33.7	3.81	0.19
	W + C	122.0	57.5	1.64	0.14	42.9	29.6	4.67	0.20
IB	W	58.9	48.3	3.11	0.22	47.6	23.9	3.85	0.13
	W + C	100.1	58.3	1.83	0.15	38.2	24.7	4.79	0.17
PW	W	124.3	58.9	1.59	0.13	156.6	57.9	1.26	0.11
	W + C	129.2	64.3	1.53	0.14	161.5	61.2	1.22	0.11
IC	W	84.0	45.7	2.88	0.19	103.7	47.0	2.33	0.16
	W + C	144.9	58.7	1.67	0.14	120.5	45.1	2.01	0.13
JH	W	118.8	47.9	2.25	0.15	—	—	—	—
	W + C	138.9	50.1	1.93	0.14	—	—	—	—
Mean	W	96.1	47.8	2.33	0.16	80.0	38.4	3.12	0.15
± s.d.		22.0	5.4	0.52	0.04	43.7	13.8	1.28	0.03
Mean	W + C	126.5	57.8	1.70	0.14	81.1	37.7	3.24	0.16
± s.d.		14.3	4.2	0.11	0.01	49.2	13.4	1.43	0.03

W – Warfarin alone, W + C – Warfarin and cimetidine

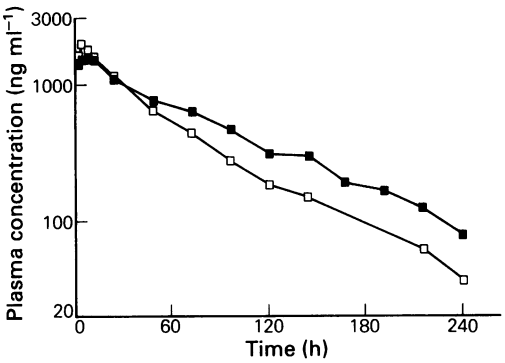

Figure 1 Plasma concentrations of R warfarin following an oral dose (15 mg) with (■) and without (□) cimetidine in a single volunteer (BH).

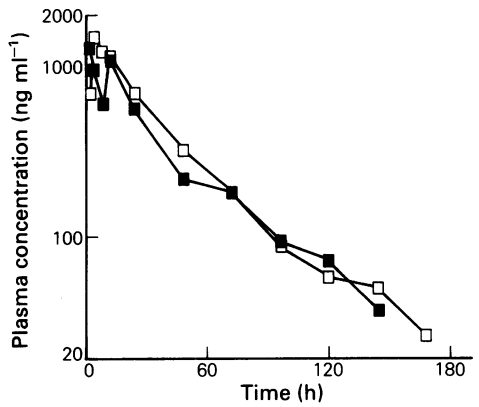

Figure 2 Plasma concentrations of S warfarin following an oral dose (15 mg) with (■) and without (□) cimetidine in a single volunteer (BH).

The results show a stereoselective pharmacokinetic interaction between cimetidine and the R enantiomer of warfarin. R warfarin is less potent than S warfarin (Breckenridge *et al.*, 1974; O'Reilly, 1974; Wingard *et al.*, 1978). Nevertheless, doses of R warfarin as low as 1 mg daily cause a measurable increase in the prothrombin time in healthy volunteers (Choonara *et al.*, paper in preparation). The significant reduction (30%) in clearance of R warfarin may explain the marked increase in prothrombin time noted in patients on racemic warfarin after the addition of cimetidine. However, a formal documentation of the pharmacodynamic consequences of the stereoselective pharmacokinetic interaction between warfarin and cimetidine is still required.

The increase in AUC and plasma half-life and the decrease in plasma clearance indicate that there is inhibition of metabolism of the R enantiomer but no consistent change in the metabolism of S warfarin. In contrast, previous studies on the stereoselective nature of warfarin drug interactions (Lewis *et al.*, 1974; O'Reilly, 1976, 1980, 1982) have shown an increase in the mean plasma half-life of S warfarin due to decreased clearance. The mean plasma half-life of the R enantiomer however has been shown to decrease (phenylbutazone and sulphinpyrazone) or remain unchanged (metronidazole and cotrimoxazole). Metronidazole and cotrimoxazole are thought to inhibit the oxidation of the S enantiomer while having no effect on R warfarin. Protein binding was not investigated in the original

Table 2 Pharmacokinetics of Vitamin K_1 and Vitamin K_1 2,3-epoxide with R and S warfarin

Volunteer		*Vitamin K_1* $t_{1/2}$ *(h)*	*Vitamin K_1* *AUC (ng ml^{-1} h)*	*Vitamin K_1, 2,3-epoxide* $t_{1/2}$ *(h)*	*Vitamin K_1, 2,3-epoxide* *AUC (ng ml^{-1} h)*
NK	R	2.95	1284	4.52	3342
	S	1.90	1183	4.02	4464
MO	R	1.89	2185	4.55	3608
	S	1.14	1884	4.97	3777
BH	R	1.18	3354	3.67	8234
	S	1.65	2206	4.51	7292
PW	R	1.85	1901	5.85	5136
	S	1.61	1600	10.45	11583
IC	R	1.99	3630	5.09	7826
	S	1.22	4258	5.03	9347
DR	R	1.09	2156	11.02	12201
	S	0.94	3623	11.14	17346

R – R-warfarin, S – S-warfarin

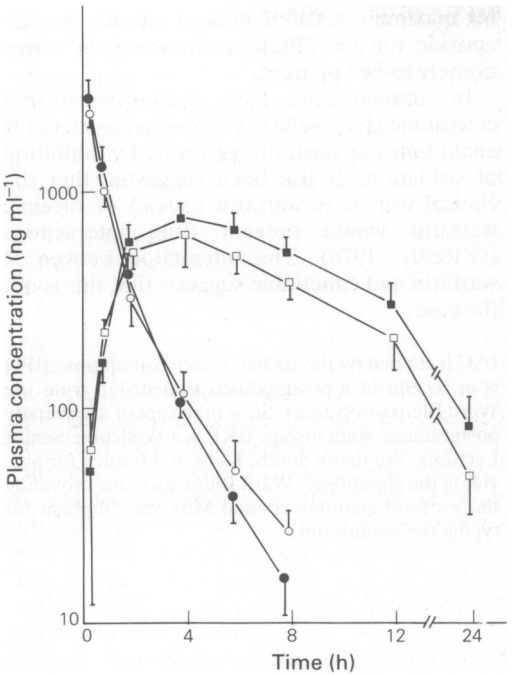

Figure 3 Mean plasma concentrations ± s.d. of vitamin K_1 (circles) and vitamin K_1 2,3-epoxide (squares), after intravenous administration of vitamin K_1 (20 mg), in volunteers on R warfarin (○, □) and S warfarin (●, ■).

Table 3 Effect of cimetidine on plasma protein binding of racemic warfarin *in vitro* at 37°C, pH 7.4.

Concentration of cimetidine ($\mu g\ ml^{-1}$)	*Free warfarin* (%)
0	1.2
0.1	1.1
1.0	1.2
10	0.9
100	1.2

sulphinpyrazone study (O'Reilly, 1982). However, it is likely that displacement occurred as this would explain both the resulting increase in clearance of R warfarin and also the increased pharmacodynamic effect observed. The phenylbutazone-interaction has been the most extensively studied (Lewis *et al.*, 1974; O'Reilly *et al.*, 1980; Banfield *et al.*, 1983) and it appears that there is a complex effect on both enantiomers involving inhibition of metabolism as well as displacement from plasma proteins.

Cimetidine is a substituted imidazole and is thus thought to be a potent inhibitor of drug oxidation (Wilkinson *et al.*, 1974; Somogyi & Gugler, 1982). This has in fact been shown to occur with antipyrine (Serlin *et al.*, 1979), diazepam (Klotz & Reimann, 1980), phenytoin (Hetzel *et al.*, 1981) and theophylline (Jackson *et al.*, 1980). Cimetidine may reduce liver blood flow (Feely *et al.*, 1981), but this is unlikely to affect warfarin which has a low extraction ratio and it is difficult to explain a stereoselective effect on this basis. There was no change in the plasma protein binding of racemic warfarin following the administration of cimetidine (Serlin *et al.*, 1979) and one would not expect any such effect as cimetidine is not highly bound to plasma proteins (13–25%) (Somogyi *et al.*, 1980). Furthermore, we have found that addition of cimetidine, at concentrations 100 fold greater than maximum therapeutic concentrations does not alter the binding of warfarin *in vitro*.

Previous work with human liver microsomes *in vitro* (Kaminsky *et al.*, 1984) has shown that each enantiomer of warfarin may undergo at least six separate cytochrome P450 mediated reactions. The major metabolites for R and S warfarin are the 6-hydroxy and the 7-hydroxy-warfarin respectively. Consistent with these findings, *in vivo* studies (Banfield *et al.*, 1983) have shown that as well as forming the 6- and the 7-hydroxywarfarin the enantiomers undergo reduction to form alcohols, and also that total recovery from urine is only 30–60% for the major metabolites. Measurement of clearance to metabolites would thus involve a formidable analytical task beyond the scope of this study. It is possible that the stereoselective effect of cimetidine may in fact be due to a regioselective effect on 6-hydroxylation as opposed to 7-hydroxylation.

The three recorded adverse drug reactions have been previously documented, although they are all uncommon.

As vitamin K_1 was administered to the volunteers to prevent prolongation of the prothrombin time, we took advantage of this opportunity to study the differential effect of the enantiomers of warfarin on vitamin K_1 metabolism. Vitamin K_1 is a co-factor for the synthesis of clotting factors II, VII, IX and X, and during this process is converted into the inactive metabolite vitamin K_1 2,3-epoxide. The epoxide is reduced back to vitamin K by a microsomal epoxide reductase and the cyclic interconversion of vitamin and epoxide is referred to as the vitamin K_1-epoxide cycle (Willingham & Matschiner, 1974; Bell, 1978). Warfarin is thought to act by inhibition of the epoxide reductase and consistent with this hypothesis we have previously demonstrated elevated levels

of vitamin K_1 2,3-epoxide following a pharmacological dose of vitamin K_1 (10 mg in patients on steady state warfarin (Choonara *et al.*, 1985).

As S-warfarin is more potent than R-warfarin (Breckenridge *et al.*, 1974; O'Reilly, 1974; Wingard *et al.*, 1978) one might have expected to see a greater accumulation of vitamin K_1 2,3-epoxide with the S isomer. Although this has been demonstrated in the rat (Schmidt *et al.*, 1977), previous work in man (Shearer *et al.*, 1977) failed to show any difference, possibly because the dose used (50 mg) exceeded that required for the maximum accumulation of vitamin K_1 2,3-epoxide. In this study we also have been unable to show a difference in epoxide formation for the enantiomers of warfarin. There was a trend towards greater AUC of the epoxide following S compared with R warfarin but this was not statistically significant ($P > 0.1$). It is probable that the dose employed in this study (15 mg) was too close to the dose required for maximum accumulation of vitamin K_1 2,3-epoxide for any difference between the enantiomers to be apparent.

In summary, we have demonstrated that cimetidine stereoselectively interacts with the R enantiomer of warfarin, probably by inhibition of oxidation. It has been suggested that the clinical use of R warfarin instead of racemic warfarin would prevent drug interactions (O'Reilly, 1976). The interaction betwen R warfarin and cimetidine suggests that this is not the case.

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