Enantiomers of warfarin and vitamin K₁ metabolism

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The effect of the individual enantiomers of warfarin at steady state (1mg daily) was investigated in five healthy volunteers. Both enantiomers produced a significant increase in prothrombin time, but the increase with S warfarin (1.8 ± 0.8 s, mean $\pm$ s.d.) was greater than with R warfarin (1.0 ± 0.3 s), despite lower steady state plasma concentrations of S warfarin, due to its more rapid clearance. Following the administration of vitamin K₁, the maximum plasma concentration and area under the plasma concentration time curve values for the metabolite vitamin K₁ 2,3-epoxide were greater after S warfarin than after R warfarin. The greater anticoagulant potency of S warfarin is reflected by a greater degree of inhibition of vitamin K₁ epoxide reductase.

Keywords warfarin vitaminK₁ stereoselectivity

Introduction

Warfarin is administered as a racemic mixture of two optically active isomers, R (+) and S(–) warfarin. These two enantiomers have different pharmacokinetic and pharmacodynamic properties. S warfarin is more potent in man, despite having a shorter half-life (Breckenridge *et al.*, 1984).

Warfarin is thought to act by interrupting the physiologically important vitamin K₁-epoxide cycle by inhibition of the enzyme vitamin K₁ 2,3-epoxide reductase (Bell & Matschiner, 1972). This results in an accumulation of the biologically inactive vitamin K₁ 2,3-epoxide which cannot be recycled back to vitamin K₁ (Ren *et al.*, 1977). Hence there is a reduction in the activity of clotting factors II, VII, IX and X.

Previous studies in man, involving single doses of the enantiomers of warfarin, have resulted in similar levels of vitamin K₁ 2,3-epoxide after both R and S warfarin following either physiological or pharmacological doses of vitamin K₁ (Shearer *et al.*, 1977; Choonara *et al.*, 1986). A stereoselective difference in coumarin-induced vitamin K₁ 2,3-epoxide formation has however been demonstrated in rats (Schmidt *et al.*, 1979; Bell & Ren, 1981). The studies in man involved

large doses of warfarin and it is possible that by the use of lower doses and also by achieving steady state concentrations, that a stereoselective effect on vitamin K₁ metabolism may be demonstrated. The aim of this study is to determine whether a stereoselective effect exists, and to determine its relationship to the anticoagulant potency of the individual enantiomers.

Method

Plan of study

Approval was obtained from the Mersey Regional Ethics Committee as well as individual consent from the five healthy volunteers who took part in the study. Each volunteer took 1 mg warfarin (R or S in a randomised order) daily for 2 weeks to allow steady state levels to be reached. They then received 10 mg vitamin K₁, diluted in 10 ml 0.9% saline, given intravenously over 10 min. They continued to take 1 mg warfarin daily for 72 h after the administration of the vitamin K₁. There was a minimum rest period of 4 weeks before the second part of the study with the other enantiomer of warfarin.

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Venous blood samples were collected prior to the warfarin and at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after the vitamin K₁. The warfarin capsules were prepared by the addition of lactose to the warfarin prior to packing gelatin capsules.

Analytical methods

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

Vitamin K₁ (the biologically active *trans*-isomer) and vitamin K₁ 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.

Calculations

The vitamin K₁ plasma concentration vs time data were analysed according to a two-compartment model using a non-linear regression

programme as described previously (Park *et al.*, 1984), to obtain the terminal half-life ($t_{1/2}$) and elimination rate constant (λ_z). The area under the plasma concentration-time curve (AUC) for vitamin K₁ 2,3-epoxide was determined by trapezoidal approximation up to 8 h. Total plasma clearance (CL) of each warfarin enantiomer was obtained by dividing the administered (1mg) dose by the steady state plasma concentration and dosing interval (24 h), assuming complete absorption. Statistical analysis was by the paired Student's *t*-test.

Results

There was no significant difference in the plasma half-life of vitamin K₁ during administration of the individual enantiomers of warfarin. Both the maximum plasma concentration ($C_{p,max}$) and the AUC for vitamin K₁ 2,3-epoxide were significantly higher ($P < 0.05$) after S warfarin than R warfarin (Figure 1). The trough steady state

Figure 1 Mean plasma concentrations ($\pm$ s.e. mean) of vitamin K₁ (circles) and vitamin K₁ 2,3-epoxide, (squares) after intravenous administration of vitamin K₁ (10 mg), in volunteers receiving R (●, ■) and S (○, □) warfarin.

Table 1 Pharmacokinetics of vitamin K₁ 2,3-epoxide and warfarin and pharmacodynamic response to warfarin

Volunteer		Vitamin K ₁ 2,3-epoxide			Warfarin		Increase in prothrombin time (s)
		$t_{1/2}$ (h)	C_{pmax} (ng ml ⁻¹)	AUC (ng ml ⁻¹ h)	Plasma concentration (ng ml ⁻¹)	CL (ml h ⁻¹ kg ⁻¹)	
DA	R	2.89	135	703	314	1.33	1.5
	S	1.76	149	900	175	2.38	2.9
RC	R	1.52	132	784	196	2.66	0.6
	S	1.52	256	1470	103	5.06	1.0
NK	R	2.48	111	549	220	2.56	0.7
	S	2.35	205	917	188	3.00	1.4
PR	R	2.63	114	747	277	2.35	1.0
	S	2.24	218	1458	176	3.70	1.1
DT	R	2.45	186	1015	235	2.16	1.0
	S	1.97	202	1140	235	2.16	2.5
Mean	R	2.39	136	760	248	2.21	0.96
	S	1.97	206*	1177*	175*	3.26*	1.78*

* $P < 0.05$

R = R warfarin, S = S warfarin

plasma concentrations of S warfarin were significantly lower due to the greater plasma clearance ($P < 0.05$) (Table 1). There was a significantly greater increase in prothrombin time with S warfarin (Table 1) ($P < 0.05$). The prothrombin time returned to within 0.2 s of its initial value (11.5–13.4 s) in all five volunteers, after the administration of vitamin K₁. The coefficient of variation for the determination of prothrombin time was 1.2%.

Discussion

The S enantiomer of warfarin has been reported to have 3.8 (Breckenridge *et al.*, 1974), 3.4 (O'Reilly, 1974) and 2.7 (Wingard *et al.*, 1978) times the potency of R enantiomer. A recent theoretical model has confirmed the greater potency of S warfarin and suggests that the effect following racemic warfarin resides almost exclusively with the S enantiomer (Chan *et al.*, 1985). If the anticoagulant activity of 4-hydroxycoumarins is related to the inhibition of epoxide reductase, then one might expect a greater accumulation of vitamin K₁ 2,3-epoxide with S warfarin. While this has been demonstrated in rats (Schmidt *et al.*, 1979; Bell & Ren, 1981), previous studies in man have failed to show any difference in epoxide formation between the two enantiomers (Shearer *et al.*, 1977; Choonara

et al., 1986). However, the doses used (50 and 15 mg respectively) may have exceeded that required for maximum accumulation of epoxide.

Since S warfarin has a greater clearance than R warfarin its steady state plasma concentrations were lower (Table 1). To compare potency, one should ideally have similar plasma concentrations. This is however very difficult to achieve because of the inter-individual variation in clearance rates. Nevertheless, the greater potency of S warfarin is emphasised, in this study, by the greater increase in prothrombin time, despite lower plasma concentrations. Furthermore, the results confirm that R warfarin does have a significant effect on clotting factor synthesis as suggested by previous studies (Breckenridge *et al.*, 1974; O'Reilly, 1974; Wingard *et al.*, 1978).

The formation of vitamin K₁ 2,3-epoxide, as represented by both C_p max and AUC, was significantly greater for the S enantiomer. As the same dose of vitamin K₁ was given, and there was no significant difference between the plasma disposition of vitamin K₁ in the presence of the enantiomers of warfarin, the formation of vitamin K₁ 2,3-epoxide may be regarded as an indicator of the degree of inhibition of epoxide reductase. The results therefore confirm the greater potency of S warfarin in man, and suggest that this greater potency may be due to a greater inhibition of epoxide reductase.

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