The plasma half-lives of the enantiomers of warfarin in warfarin-resistant and warfarin-susceptible rats

Warfarin as used rodenticidally and clinically is a racemic mixture of two enantiomers. In normal male rats (−)-warfarin is about six times more potent as an anticoagulant than (+)-warfarin (Eble, West & Link, 1966; Breckenridge & Orme, 1972). Also in normal male rats (−)-warfarin is eliminated from the plasma at about half the rate of (+)-warfarin (Breckenridge & Orme, 1972). I have examined the warfarin enantiomers in both normal and warfarin-resistant rats of either sex.

Rats (average weight 220 g) homozygous for warfarin resistance (SH strain) and normal warfarin-susceptible Sprague-Dawley rats were used. Phenobarbitone-pretreatment comprised five consecutive daily injections of phenobarbitone sodium (75 mg/kg); the last injection was made approximately 24 h before warfarin injection. Blood samples (obtained by aortic puncture) were citrated and then centrifuged to obtain the plasma. Plasma prothrombin times were determined by the method of Quick (1957). Plasma warfarin was determined by the method of Corn & Berberich (1967).

Table 1 shows that in each sex of both resistant and non-resistant rats (+)-warfarin was eliminated from the plasma two to three times faster than (−)-warfarin. The plasma half-lives of the respective enantiomers were almost identical in male resistant and male non-resistant rats. The half-lives of the respective enantiomers in female resistant rats were about two-thirds of those in female non-resistant rats. The apparent volumes of distribution of the enantiomers were similar in both rat strains (results not shown). Also similar in both strains was the lower rate of elimination of the enantiomers by female rats.

However, Table 1 shows significant differences between the strains in the susceptibility to the anticoagulant action of warfarin. At 31 h after warfarin administration the prothrombin times of the resistant rats were hardly affected while those of the non-resistant rats were markedly prolonged. The prothrombin times show in normal male and female rats that (−)-warfarin was more potent than (+)-warfarin. In a

Table 1. *Comparison of the plasma half-lives and anticoagulant efficacies of the enantiomers of warfarin in warfarin-resistant and non-resistant rats.* The warfarin enantiomers (sodium salts) were dissolved in water and injected intraperitoneally (5 mg/kg). Prothrombin times were determined immediately after and 31 h after drug administration. The same batches of reagents were used throughout. Results are given as the mean of 3 or more animals with ranges in parentheses. Plasma warfarin concentrations for the calculation of half-lives were measured at 2, 8, 24 and 31 h. Half-lives and prothrombin times for warfarin-resistant and non-resistant rats of the same sex were determined at the same time during the same experimental period.

Strain	Sex	Enantiomer	Half-life (h)	Prothrombin time (s) 0 h	31 h
SH (resistant)	Male	(+)	7·3	13 (12·5–13)	13 (12·5–13)
		(−)	12	13 (12·5–13)	14 (13–15)
	Female	(+)	9	11 (10–12)	12 (12–12·5)
		(−)	23	11 (10–12)	14 (12–17)
Sprague-Dawley (non-resistant)	Male	(+)	7·6	14 (13–14)	23·5 (17–30)
		(−)	11	14 (13–14)	97·5 (80–115)
	Female	(+)	11·6	12 (11·5–13)	34 (28–45)
		(−)	33	12 (11·5–13)	90 (86–95)

separate experiment the potency ratio in normal female rats was found to be 6·8. The 5 mg/kg doses of (+)- or (−)-warfarin were too small to show a clear difference in anticoagulant potencies in terms of prolongation of prothrombin times in resistant rats. However, thrombotest (Owren, 1959) determinations (which give a more sensitive indication of the anticoagulant action of warfarin; Tat & Lewis, 1962) made 31 h after warfarin administration, clearly indicated that (−)-warfarin was also a more potent anticoagulant than (+)-warfarin in resistant rats.

Phenobarbitone-pretreatment of non-resistant rats did not markedly affect the stereoselectivity of elimination of the enantiomers, although the half-lives of the enantiomers were markedly decreased (to 2·5 h and 4·5 h for (+)- and (−)-warfarin respectively in male rats; and 4·6 h and 8·4 h for (+)- and (−)-warfarin respectively in female rats).

This work, indicating that warfarin-resistant and warfarin-susceptible rats eliminate the respective enantiomers of warfarin at similar rates, supports the conclusion of previous workers using racemic warfarin (Pool, O'Reilly & others, 1968; Hermodson, Suttie & Link, 1969; Davis & Davies, 1970) that a faster rate of warfarin metabolism and excretion is not mainly responsible for warfarin resistance. It is unlikely that the approximately 30% higher rate of plasma elimination observed in the present study in warfarin resistant females compared with non-resistant female rats would contribute significantly to warfarin resistance.

The Corn & Berberich (1967) assay for measuring plasma warfarin used in the present paper has been criticized (O'Reilly & Aggeler, 1970) on the basis that fluorescent warfarin metabolites may interfere. However, my plasma elimination data for non-resistant male rats and the conclusions drawn from them do not differ markedly from those of Breckenridge & Orme (1972) obtained using an assay procedure (Lewis, Ilnicki & Carlstrom, 1970) designed to be specific for warfarin.

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