J. Pharm. Pharmacol. 1990, 42: 861–866
Received June 21, 1990

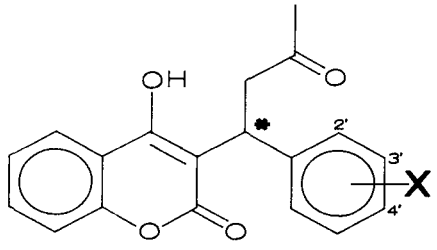
Enantioselective Structure-pharmacokinetic Relationships of Ring Substituted Warfarin Analogues in the Rat

L. G. M. BAARS, M. T. SCHEPERS, J. J. R. HERMANS, H. J. J. DAHLMANS AND H. H. W. THIJSSEN

Dept of Pharmacology, University of Limburg, P.O. Box 616, 6200 MD Maastricht, The Netherlands

Abstract—The enantiomer specific pharmacokinetics of ring substituted warfarin analogues have been studied in the rat after the administration of 2 mg kg^{-1} of the racemates. The stereoselective differences observed were due to stereoselective plasma protein binding and stereoselective intrinsic hepatic clearance. Greater binding was observed for the *S*-enantiomers except for 2′-substituted analogues where the *R*-enantiomers were more tightly bound. The stereoselectivity in the binding ranged up to a factor of about 4. All substituted warfarins showed a higher intrinsic clearance than warfarin. Enantiomer selectivity depended on the position of the substituent; warfarin and 3′-substituted analogues showed $R > S$; 4′- and 2′ substituted warfarins showed $S > R$ stereoselectivity. Exceptions to this generality were seen for 4′-methoxy- and 4′-methylwarfarin which did not show stereoselective hepatic clearance.

Stereoselective differences in drug disposition and drug activity have received increasing attention as awareness has grown that pharmacokinetic and pharmacodynamic descriptions of a drug applied as a racemic mixture are meaningless unless the fate of the individual enantiomers is established (Ariëns 1984; for reviews see Walle & Walle 1986; Evans et al 1988; Tucker & Lennard 1990). The closely related 4-hydroxycoumarin anticoagulants warfarin and acenocoumarol (4′-nitrowarfarin) are in use as racemic mixtures. The stereoselectivity of the pharmacokinetics and the pharmacodynamics of their enantiomers in man and rat has been widely studied (Breckenridge et al 1973; Yacobi & Levy 1974; Toon et al 1986; Godbillon et al 1981; Thijssen et al 1985, 1986). In man the *S*-enantiomers of both compounds are eliminated more rapidly than the *R*-enantiomers; the differences are modest for the warfarin enantiomers but 10–20 fold for the enantiomers of acenocoumarol. The body clearance of the acenocoumarol enantiomers is at least 100 times that of *R*/*S*-warfarin (Godbillon et al 1981; Thijssen et al 1986). Those differences between warfarin and acenocoumarol are also observed in the rat. However, in the rat, *R*-warfarin is eliminated faster than *S*-warfarin. The stereoselectivity of acenocoumarol elimination in the rat is comparable to that of man, i.e. *S*-acenocoumarol is cleared 3–4 times faster (Thijssen et al 1985). The effects of the introduction of the 4′-nitro substituent on clearance and stereoselectivity prompted us to investigate the disposition of a series of phenyl-substituted warfarin (I) analogues in the rat. The compounds were administered as their racemates and the individual enantiomers were analysed by a stereoselective HPLC procedure. Stereoselectivity in plasma protein binding was accounted for in the pharmacokinetic evaluation.



I. The structure of warfarin (X = H) and the ring substituted analogues. The chiral centre is indicated by *.

Materials and Methods

Drugs

R, *S*-Warfarin was obtained from Sigma Chemicals (St Louis, USA); *R*, *S*-4′-chlorowarfarin was from Aldrich (Brussels, Belgium); *R*,*S*-4′-nitrowarfarin was a gift from Ciba-Geigy (Arnhem, the Netherlands). Optically pure isomers of warfarin, 4′-chlorowarfarin and 4′-nitrowarfarin were at our disposal (Hermans & Thijssen 1990). Phenyl ring-substituted warfarin analogues were synthesized in our laboratory according to standard procedures. Briefly, substituted benzalacetones were prepared from the respective benzaldehydes by condensation with acetone under alkaline conditions in an ethanol–water mixture. The benzalacetones were purified by crystallization (TLC was used to monitor purity) and identified by mass spectrometry (GC-MS, Hewlett Packard 5995). Conjugation of the benzalacetone with 4-hydroxycoumarin via Michael addition was by refluxing an equimolar mixture of the reactants in methanol as described by Bush & Trager (1983). The compounds were purified by crystallization (TLC and HPLC were used for analysis). GC-MS identification of the warfarins was performed after derivatization with diazomethane. The acidity (pK_a) of the warfarins was estimated from the pH-dependent change in UV (272 nm) absorption of 10 $\mu g\ mL^{-1}$ solutions in buffers (pH range 2–11, ionic strength 0·5).

Drug analysis

Total drug concentrations, i.e. the *R*-+*S*-enantiomer, in samples were assayed by reversed phase HPLC based on techniques described by Thijssen et al (1985). For each compound the eluent was adapted for optimal resolution and

Correspondence to: H. H. W. Thijssen, Department of Pharmacology, University of Limburg, PO Box 616, 6200 MD Maastricht, The Netherlands.

sensitivity. A Chromspher C18 column, 3 × 100 mm (Chrompack, Middelburg, The Netherlands) was used. The drugs were extracted from the samples with light petroleum-dichloromethane (1:1) at pH 4·4 using one of the enantiomers of either warfarin, 4′-chlorowarfarin, or 4′-nitrowarfarin as internal standard. The choice depended on the chromatographic behaviour in relation to the compound to be assayed. Following evaporation of the extract the residue was dissolved in 50 μL of acetonitrile and 10 μL of this was injected. Total concentration was estimated using peak area ratios. The limit of detection, using 0·08 mL of rat plasma, was 0·03 – 0·05 μg mL^{-1}. The ratio of the *R*- and *S*-enantiomers in the samples was estimated by the procedure of Banfield & Rowland (1983); to the remaining 40 μL of the extract was added 20 μL of a solution of 10 mg mL^{-1} dicyclohexanecarbodiimide and 20 μL of a solution of 10 mg mL^{-1} *N*-carbobenzyloxy-L-proline, both in acetonitrile. Extracts of blank samples with added racemate were run in parallel. The reaction was completed at 40°C overnight. The diastereomeric esters were separated by normal phase chromatography using a Chromspher Si Column, 3 × 100 mm, and mixtures of hexane–THF–0·1% acetic acid as eluting solvent. The esters of the *R*- and *S*-enantiomers were completely separated, with the ester of the *S*-enantiomer eluting first. The ratio of the *R*- and *S*-enantiomer in the sample was calculated from the peak areas of the sample *R*- and *S*-peak in relation to the ratio of the racemic reference. The concentrations of the individual enantiomers in a sample were calculated from the *R*/*S* ratio and the total concentration.

Partition coefficients

The partition coefficients of the warfarin analogues for the n-octanol water system at pH 7·4 was estimated as follows. To 1 mL 0·1 M phosphate buffer, pH 7·4 saturated with *N*-octanol, was added 20 μg of the compound (20 μL of a 1 mg mL^{-1} pH 8–10 aqueous stock solution). Three 0·3 mL samples were equilibrated with 0·3 mL n-octanol saturated with buffer by shaking for 30 min. The concentrations in the aqueous phase were assayed by HPLC.

Plasma protein binding

Plasma protein binding was determined by equilibrium dialysis. Rat plasma pooled from six rats was used. Dialysis bags (diameter 6·4 mm) were filled with 1 mL plasma containing 15 μg of the racemate. Dialysis was performed against 4 mL of 0·066 M phosphate buffer, pH 7·4 containing 0·01 % sodium azide in screw stoppered 5 mL flasks. The flasks were gently tumbled overnight at room temperature (22°C). Assay of the enantiomers in the buffer compartment and the plasma compartment were performed as described above.

Animal experiments

Male Wistar rats, 250–300 g, were used and had free access to labfood and water. Under light ether anaesthesia the rats were fitted with a catheter (PE 10) in the left femoral artery. The catheter was exteriorized in the neck. The animals were allowed to recover at least two days before use. The warfarin analogues, as racemates (1 mg mL^{-1} saline, pH 7–8), were administered subcutaneously in 2 mg kg^{-1} amounts. Blood samples, 200 μL, were taken at regular times and collected on 22 μL 0·1 M trisodium citrate in Eppendorf cuvettes. Plasma was prepared immediately and stored at −20°C until analysed.

Pharmacokinetic analyses

The plasma half-life $t_{\frac{1}{2}}$ was estimated from the terminal elimination phase by linear regression. The area under the plasma concentration-time curve was calculated by the trapezoidal rule with the addition of the extrapolated part; $AUC_t + C_t.t_{\frac{1}{2}}/\ln 2$, where AUC_t is the area from t = 0 to the last concentration C_t estimated. Total plasma clearance CL, assuming complete absorption of the dose D, was calculated by D/AUC, the apparent volume of distribution V_{area} by $CL.t_{\frac{1}{2}}/\ln 2$. The pharmacokinetic constants for the unbound fraction were obtained by correcting for the protein binding; e.g. $CL_u = CL/f_u$, where f_u is the unbound fraction.

Statistical evaluation

Enantiomeric differences were tested for their significance by two-tailed paired *t*-tests.

Results

The warfarin analogues investigated are listed in Table 1 together with some physicochemical parameters. The pKa values of the compounds did not differ grossly, ranging between 4·8 and 5·1, indicating no influence of the substituents on the acidity of the 4-hydroxy group of the coumarin nucleus. For warfarin and 4′-nitrowarfarin (acenocoumarol) our data are in agreement with those of Van der Giesen (1982).

The partition coefficient varied with the lipophilicity of the substituent. Except for 2′-methoxywarfarin, the correlation of $\log(P_X/P_H)$ with Hansch's π_X parameter was good indicating that the substituents did not alter the molecular conformation in such a manner that deviations of the additivity of the fragmental lipophilicity occur (Hansch & Leo 1979).

The pharmacokinetic constants of the warfarin analogues are presented in Table 2 and examples of the impact of substituents on the plasma elimination are shown in Fig. 1.

Table 1. pK_a and Log P constants of ring substituted warfarins.

Compound	pK_a	log P	$\log P_X - \log P_H$	π_X
Warfarin	5·0	1·20	0·00	0·00
4′-Nitro	5·0	0·98	−0·22	−0·28
3′-Nitro	4·9	0·92	−0·28	−0·28
4′-Cyano	nd	0·64	−0·56	−0·57
4′-Chloro	4·9	2·03	0·83	0·71
3′-Chloro	5·0	2·00	0·80	0·71
2′-Chloro	5·1	2·02	0·82	0·71
4′-Bromo	5·0	2·24	1·12	0·86
3′-Bromo	nd	2·24	1·04	0·86
4′-Methyl	4·9	1·71	0·51	0·56
3′-Methyl	nd	1·73	0·53	0·56
2′-Methyl	nd	1·92	0·72	0·56
4′-Methoxy	5·1	1·25	0·05	−0·02
3′-Methoxy	5·0	1·20	0·00	−0·02
2′-Methoxy	5·2	1·59	0·39	−0·02

pK_a and log P values were determined as described in the method section. nd = not determined. Hansch π_X constants are obtained from Hansch & Leo (1979). log P_X-log P_H relate to π_X by; $1{\cdot}058 \times \pi_X + 0{\cdot}07$, $r = 0{\cdot}969$, $P < 0{\cdot}001$.

Table. 2. Pharmacokinetic constants of *R*/*S*-ring substituted warfarins in the rat.

X		$t_{\frac{1}{2}}$ (h)	AUC (μg h mL^{-1})	CL (mL h^{-1} kg^{-1})	V_{area} (mL kg^{-1})
H	*R*	4·75±0·29**	32·0±4·0**	32·3±4·2**	215±14·7*
	S	8·75±0·43	92·2±4·3	10·9±0·5	135±0·6
4′-NO_2	*R*	1·70±0·10**	12·6±0·7**	80·1±4·6**	193±4·3
	S	0·45±0·05	3·5±0·2	288·6±21·0	184±7·2
3′-NO_2	*R*	2·9±0·3*	22·4±3·3**	49·0±6·6**	195±15
	S	5·2±0·8	47·9±8·6	26·2±6·5	167±19
4′-CN	*R*	7·8±0·76**	39·2±3·2**	25·9±2·2**	295±54*
	S	0·97±0·17	7·1±1·5	156±37	197±8
4′-Cl	*R*	1·96±0·14**	11·9±1·4	88·0±9·3	239±11*
	S	1·22±0·07	10·4±1·4	103·0±14	176±38*
3′-Cl	*R*	2·47±0·07*	5·3±0·1**	190·0±3·7**	669±12·7**
	S	7·8±1·4	25·4±4·2	41·3±6·2	436±7
2′-Cl	*R*	28·3±5·9**	455·0±103**	2·4±0·4**	89±1·4*
	S	3·5±1·0	42·5±12·2	27·1±0·3	116±6·1
4′-Br	*R*	2·58±0·17**	30·5±4·4*	34·4±5·7**	124±12·2*
	S	1·30±0·14	14·4±1·4	70·7±7·5	128±1·7
3′-Br	*R*	1·66±0·13**	7·5±0·3**	132·5±5·5	314±14
	S	8·16±0·35	45·2±1·8	22·2±0·9	259±16
4′-CH_3	*R*	1·43±0·13	9·1±0·6*	111·0±6·6*	226±8·8**
	S	1·62±0·12	15·4±1·1	65·7±4·4	152±8·8
3′-CH_3	*R*	1·56±0·14**	8·8±0·6**	114·0±8·0**	252±10·3*
	S	7·3±2·7	57·8±16	21·6±5·8	177±6
2′-CH_3	*R*	11·0±1·5**	112·2±14·6**	9·0±1·2**	140±1*
	S	3·4±0·12	25·6±4·9	40·6±7·8	194±30
4′-OCH_3	*R*	1·51±0·2	14·3±1·5	71·3±6.8	149±5*
	S	1·64±0·2	18·8±1·8	54·3±5·6	121±4
2′-OCH_3	*R*	2·9±0·2**	16·4±1·8*	62·5±6·4*	258±25*
	S	6·0±0·14	46·4±5.0	22·0±2·3	188±16
2′-OCH_3	*R*	16·0±0·6**	177·0±12·6**	5·7±0·4**	130±4·7**
	S	2·5±0·3	16·3±1·4	62·2±5·4	215±7

The data are the mean±s.e.m. of 3–4 rats. *$P<0·05$, **$P<0·01$.

Table 3 presents the plasma protein binding of the compounds as well as the pharmacokinetic constants of the unbound fraction, i.e. unbound clearance (=intrinsic clearance) and free volume of distribution.

There is a significant correlation between log P and log (bound/free); *R*: log B/F=0·337 log P+1·626, r=0·711, $P<0·01$ (n=12); *S*: log B/F=0·380 log P+1·752, r=0·797, $P<0·01$ (n=12).

N.B: the 2′-substituted derivatives have not been included in the correlations because their mechanism of interaction with plasma proteins may be different, i.e. inversion of binding stereoselectivity (Table 3; see also Discussion).

All compounds affected the blood clotting activity (thrombotest value). Fig. 2 shows the effect of responses of the experiments of Fig. 1. Compared with racemic 4′-nitrowarfarin the effect of racemic warfarin is more intense and longer lasting, consistent with a longer residence time of the latter. The same can be argued for racemic 3′-chlorowarfarin in comparison with the other chloro substituted analogues (Fig. 2B). Remarkably, 2′-chlorowarfarin notwithstanding the long half-life of the *R*-enantiomer showed only weak anticlotting activity. This suggests the intrinsic activity of *R*-2′-chlorowarfarin to be weak. Also the other 2′-substituted warfarins showed weak activity. As the change in prothrombin complex activity (PCA) following the administration of the racemic compounds is a function of the individual enantiomeric pharmacokinetics and pharmacodynamics, a comparative quantitative evaluation of the effects of the racemates is not possible from our experiments.

Discussion

The present study with a series of warfarin analogues was undertaken to establish the influence of substituents on pharmacokinetics, prompted by the observation that the introduction of a 4′-nitro group (acenocoumarol) greatly enhances the metabolic clearance of both the enantiomers, enhances the enantioselective pharmacokinetics, and reverses the stereoselective elimination in the rat (Godbillon et al 1981; Thijssen et al 1985, 1986). The data for *R*/*S*-warfarin and *R*/*S*-4′-nitrowarfarin are comparable to those obtained in single enantiomer studies indicating the absence of mutual interactions. This has also been observed in man (Banfield et al 1983; Toon et al 1986; Gill et al 1988).

As the warfarin analogues have in common a relatively high lipophilicity (Table 1) and a high plasma protein binding (Table 3) it is reasonable to assume their elimination, like warfarin and 4′-nitrowarfarin, to be solely by hepatic

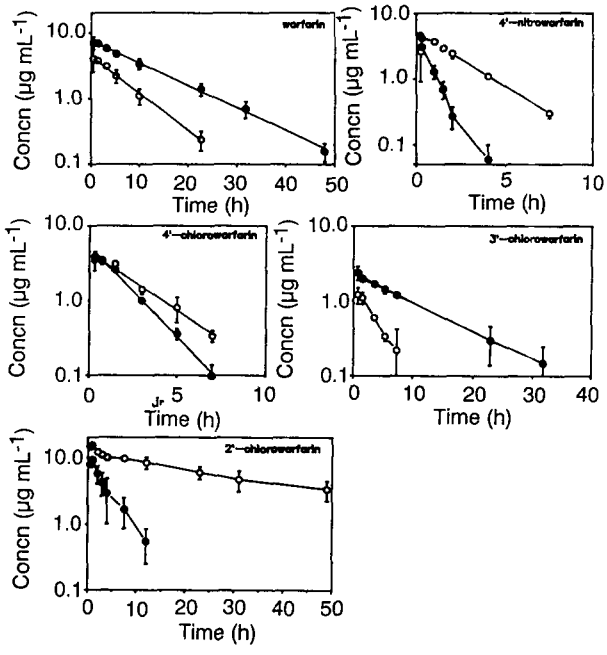


FIG. 1. The plasma concentration time curves of the enantiomers of warfarin, 4′-nitrowarfarin, and 2′-, 3′-, 4′-chlorowarfarin after their subcutaneous administration (2 mg kg^{-1}) to rats. The curves are the mean ±s.d. of 3–4 rats. *R*-enantiomer, O——O; *S*-enantiomer, ●——●.

metabolism. With this assumption the warfarin analogues tested can be classified as low clearance drugs; i.e. their plasma clearance is much less than the liver flow. Therefore, their plasma pharmacokinetics are determined by plasma protein binding and intrinsic hepatic clearance (Wingard et al 1978; Rowland & Tozer 1980). This study shows both parameters to be determining factors for the stereoselective pharmacokinetics of the compounds; the stereoselective differences in protein binding are consistent with the differences observed for V_{area}; e.g. for all the racemates it holds that the enantiomer having the highest protein binding shows the lowest V_{area} and the highest peak concentration. However, there was no significant correlation between the protein binding and V_{area}; r = 0·166 and −0·32 ($P > 0·05$) for the *R*- and *S*-enantiomers, respectively. In comparison with warfarin, the introduction of substituents greatly enhanced the intrinsic clearance, and in most cases also enhanced the stereoselectivity of the intrinsic clearance. The greatest differences between the enantiomers were observed for 4′-cyano and 4′-nitrowarfarin (Table 3). For some compounds the intrinsic clearance greatly exceeded the liver plasma flow (about 2 L h^{-1}kg^{-1}; Daemen et al 1989) of the rat. The strong protein binding, however, makes them low clearance drugs.

Remarkably, depending upon the position of the substituent, inversions in enantioselectivity of the protein binding as well as of the intrinsic clearance were observed. Warfarin and the 4′- and 3′-substituted analogues showed greater plasma protein binding for the *S*-enantiomer, but the opposite was true for the 2′-substituted analogues. Furthermore, the ratio in the enantioselective binding was high for the 2′-substituted warfarins. Recent studies, using stop-flow techniques, have presented evidence for a three-step process in the warfarin-albumin interaction, a rapid association

Table 3. Plasma protein binding[a] and pharmacokinetic constants[b] of the unbound fraction of substituted warfarins in the rat.

X		fu (×100)	CL/fu(mL h^{-1} kg^{-1})	V_{area}/fu (mL kg^{-1})
H	*R*	2·26±0·06*	1429	9513
	S	1·12±0·03	973	12053
4′-NO_2	*R*	1·22±0·06*	6557	15819
	S	0·94±0·04	30702	19574
3′-NO_2	*R*	0·64±0·01*	7597	30233
	S	0·44±0·02	5954	37954
4′-CN	*R*	1·16±0·05*	2223	25322
	S	0·83±0·04	18795	23734
4′-Cl	*R*	0·77±0·06*	11486	31201
	S	0·55±0·04	18798	32116
3′-Cl	*R*	0·72±0·07*	26462	93175
	S	0·30±0·03	13766	145333
2′-Cl	*R*	0·14±0·02*	1655	61725
	S	0·91±0·12	2978	12725
4′-Br	*R*	0·24±0·02ns	14156	51234
	S	0·22±0·01	32431	58532
3′-Br	*R*	0·29±0·01*	45689	108138
	S	0·14±0·01*	15524	181119
4′-CH_3	*R*	0·57±0·01*	19542	39788
	S	0·40±0·01	16507	38090
3′-CH_3	*R*	0·64±0·10*	17868	39498
	S	0·32±0·02	6603	55660
2′-CH_3	*R*	0·29±0·03*	3125	38611
	S	1·14±0·02	3561	17017
4′-OCH_3	*R*	0·79±0·04*	9048	18947
	S	0·60±0·04	9050	20166
3′-OCH_3	*R*	0·87±0·06*	7159	29553
	S	0·66±0·05	3333	28485
2′OCH_3	*R*	0·20±0·02*	2850	64850
	S	0·69±0·03	9014	31159

[a] The unbound fractions fu are the mean ± s.e.m. of 4 estimates in pooled rat plasma. [b] Unbound hepatic clearance and unbound apparent volume of distribution are calculated from the data of Table 2. *$P < 0·01$; ns = not significant.

followed by a rate-limiting translocation of the drug to a binding site inside a U-shaped cleft where the final binding complex is formed (Bos et al 1989). The restricted rotation of the *ortho*-substituted phenyl ring would favour the positioning of the *R*-configuration in that cleft. The stronger binding of the *S*-enantiomers of warfarin and 4′-nitrowarfarin to rat

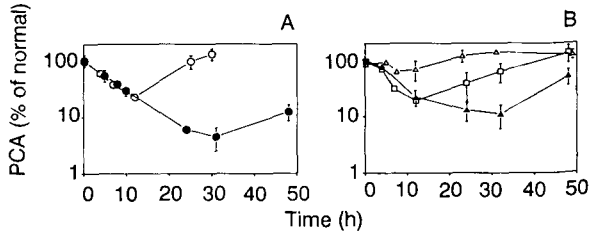


FIG. 2. The response of the plasma prothrombin complex activity (PCA) in the experiments described in Fig. 1. A: warfarin, ●——●; 4′-nitrowarfarin, O——O. B: 2′-chlorowarfarin, △——△; 3′-chlorowarfarin, ▲——▲; 4′-chlorowarfarin, □——□.

plasma protein has been shown with ratios of unbound fraction of about 1·6 (Yacobi & Levy 1977) and 1·1 (Thijssen et al 1985). In human plasma, *S*-warfarin binding is stronger (Yacobi & Levy 1977). Inversion in enantioselectivity of protein binding has recently been reported for the *R/S*-warfarin and *R/S*-acenocoumarol binding to human plasma albumin; the greater binding, assessed by affinity chromatography, was observed for *S*-warfarin and for *R*-acenocoumarol (Fitos et al 1989). Binding to α-acid glycoprotein has been reported for some drugs to have opposite stereoselectivity compared with albumin (Walle et al 1983; Fitos et al 1989). However, as the 4-hydroxycoumarins are weak acids, the contribution of α-acid glycoprotein, which binds mainly cationic drugs, will be of minor importance (Urien et al 1986).

Warfarin and 4′-nitrowarfarin biotransformation has been shown to involve mainly the hydroxylation of the coumarin structure (Fasco et al 1976; Pohl et al 1976; Dieterle et al 1977; Toon et al 1986; Thijssen & Baars 1987). With the exception of the methoxy and probably the methyl derivatives (see later), this will probably be true also for the warfarin analogues we studied. Recent in-vitro studies with rat liver microsomes have indicated that the clusters of cytochrome P450 isoenzymes involved in *R/S*-warfarin and *R/S*-4′-nitrowarfarin biotransformation may differ from each other explaining the inversion in the stereoselectivity in hepatic clearance (Hermans & Thijssen 1990). This may also hold for the other derivatives. Nevertheless, a general pattern in intrinsic clearance of metabolism is evident; $S > R$ for the 4′-substituted derivatives and $R > S$ for the 3′-substituted. To explain the inversion in enantioselectivity between warfarin and phenprocoumon in rat microsomes, Heimark & Trager (1984) postulated the hemiketal of *R*-warfarin to be topographically similar to *S*-phenprocoumon at the active site of cytochrome P450. This is not supported by the present study, and the differences have to be sought in the variety of cytochrome P450 isoenzymes expressing a broad spectrum of binding and reaction selectivity (Testa 1988). The absence of stereoselectivity in intrinsic clearance of metabolism for 4′-methoxy and 4′-methylwarfarin may indicate that their main route of metabolism, i.e. *O*- demethylation for 4′-methoxywarfarin (own observation) and methyl hydroxylation for 4′-methyl are not stereoselective.

The V_{area} of the unbound fraction for some compounds suggests stereoselective tissue binding. However, as the distribution volumes of the analogues are small, 10–30% of the body mass (Table 2), and mainly occupy the albumin space, the estimated unbound distribution volume is merely the result of the mathematical operation (Rowland & Tozer 1980). The 3′-chloro derivative might be an exception; V_{area} values observed of 669 and 436 mL kg^{-1} for the *R*- and *S*-enantiomers, respectively. We have no explanation for these relatively high distribution volumes.

In summary, the study affirms stereoselectivity in plasma protein binding and in intrinsic hepatic clearance to be determining factors in the enantioselective pharmacokinetics of low clearance drugs. For the warfarin analogues, the stereoselectivity in both processes was up to a factor 3–4 for protein binding and 8–9 for the intrinsic metabolic clearance. Small structural changes, i.e. the position of the substituent, may invert the stereoselectivity. The latter observation shows that care should be taken in predicting the enantiomer-specific pharmacokinetics of a racemate from data of closely related racemates.

References

Ariëns, E. J. (1984) Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. Eur. J. Clin. Pharmacol. 26:663–668

Banfield, C., Rowland, M. (1983) Stereospecific high-performance liquid chromatographic analysis of warfarin in plasma. J. Pharm. Sci.72: 921–924.

Banfield, C., O'Reilly, R., Chan, E., Rowland, M. (1983) Phenylbutazone-warfarin interaction in man: further stereochemical and metabolic considerations. Clin. Pharmacol. Ther. 16: 669–675

Bos, O. J. M., Fischer, M. J. E., Wilting, J., Janssen, L. H. M. (1989) Mechanism by which warfarin binds to human serum albumin. Biochem. Pharmacol. 38: 1979–1984

Breckenridge, A. M., Orme, M., Wessling, H., Lewis, R. J., Gibbons, R. (1973) Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clin. Pharmacol. 15: 424–430

Bush, E., Trager, W. F. (1983) High-yield synthesis of warfarin and its phenolic metabolites: new compounds. J. Pharm. Sci. 72: 830–831

Daemen, M. J. A. P., Thijssen, H. H. W., Van Essen, H., Vervoort-Peters, H. T. M., Prinzen, F. W., Struyker Boudier, H. A. J., Smits, J. F. M. (1989) Liver blood flow measurement in the rat. J. Pharmacol. Meth. 21: 287–297

Dieterle, W., Faigle, J.W., Sulc, M., Theobald, W. (1977) Biotransformation and pharmacokinetics of acenocoumarol (Sintrom). Eur. J. Clin. Pharmacol. 11: 367–375

Evans, A. M., Nation, R. L., Sansom, L. N., Boehner, F., Somogyi, A.A. (1988) Stereoselective drug disposition: potential for misinterpretation of drug disposition data. Br. J. Clin. Pharmacol. 26: 771–780

Fasco, M.J., Baker, F.D., Fenton, J. W. (1976) Warfarin: stereochemical aspects of its metabolism by rat liver microsomes. Biochem. Pharmacol. 25: 2153–2162

Fitos, I., Visy, J., Magyar, A., Kajtar, J., Simonyi, M. (1989) Inverse stereoselectivity in the binding of acenocoumarol to human serum albumin and to alpha-1 acid glycoprotein. Ibid. 38: 2259–2262

Gill, T. S., Hopkins, K. J., Rowland, M. (1988) Stereospecific assay of nicoumalone: application to pharmacokinetic studies in man. Br. J. Clin. Pharmacol. 25: 591–598

Godbillon, J., Richard, J., Gerardin, H., Meinert, T., Kasper, W., Jahnchen, E. (1981) Pharmacokinetics of the enantiomers of acenocoumarol in man. Ibid. 5: 621–629

Hansch, C., Leo, A. (1979) Substitution Constants for Correlation Analysis in Chemistry and Biology. Wiley Intersci. New York

Heimark, L.D., Trager, W.F. (1984) The preferred solution conformation of warfarin at the active site of cytochrome P450 based on the CD spectra in octanol/water model system. J. Med. Chem. 27: 1092–1095

Hermans, J. J. R., Thijssen, H. H. W. (1990) Comparison of the rat liver microsomal metabolism of the enantiomers of warfarin and 4′-nitrowarfarin (acenocoumarol). Xenobiotica (in press)

Pohl, L. H., Bales, R., Trager, W. F. (1976) Warfarin: stereochemical aspects of its metabolism in vivo in the rat. Res. Commun. Chem. Pathol. 15: 233–255.

Rowland, M., Tozer, T. N. (1980) Clinical Pharmacokinetics. Lea and Febiger, Philadelphia

Testa, B. (1988) Substrate and product stereoselectivity in monooxygenase-mediated drug activation and inactivation. Biochem. Pharmacol. 37: 85–92

Thijssen H. H. W., Baars, L. G. M. (1987) The biliary excretion of acenocoumarol in the rat: stereochemical aspects. J. Pharm. Pharmacol. 39: 655–657

Thijssen, H. H. W., Baars, L. G. M., Drittij-Reijnders, M. J. (1985) Stereoselective aspects in the pharmacokinetics and pharmacodynamics of acenocoumarol and its amino and acetamido derivatives in the rat. Drug Metab. Dispos. 13: 593 597

Thijssen, H. H. W., Janssen, G. M. J., Baars, L. G. M. (1986) Lack of effect of cimetidine on pharmacodynamics and kinetics of single

oral dose of R- and S-acenocoumarol. Eur. J. Clin. Pharmacol. 30: 619–623

Toon, S., Lon, L. K., Gibaldi, M., Trager, W. F., O'Reilly, R. A., Motley, C. H., Goulart, D. A. (1986) The warfarin-sulfinpyrazone interaction: stereochemical considerations. Clin. Pharmacol. Ther. 39: 15–24

Tucker, G. T., Lennard, M. S. (1990) Enantiomer specific pharmacokinetics. Pharmacol. Ther. 45: 309–329

Urien, S., Albengres, E., Pinquier, J.-L., Tillement, J.-P. (1986) Role of alpha-1 acid glycoprotein, albumin, and non-esterified fatty acids in serum binding of apazone and warfarin. Clin. Pharmacol. Ther. 39: 683–689

Van der Giesen, W. F. (1982) Physicochemical behaviour of some 4-hydroxycoumarins, especially the binding of warfarin to human serum albumin. Ph.D. thesis, University of Utrecht, The Netherlands

Walle, U. K., Walle, T., Bai, S. A., Olanoff, L. S. (1983) Stereoselective binding of propranolol to human plasma, alpha-1 acid glycoprotein and albumin. Clin. Pharmacol. Ther. 34: 718–723

Walle, T., Walle, U. K. (1986) Pharmacokinetic parameters obtained with racemates. Trends Pharmacol. Sci. 7: 155–158

Wingard, L. B., O'Reilly, R. A., Levy, G. (1978) Pharmacokinetics of warfarin enantiomers: a search for intrasubject correlations. Clin. Pharmacol. Ther. 23: 212–217

Yacobi, A., Levy, G. (1974) Comparative pharmacokinetics of coumarin anticoagulants. XII. Pharmacokinetics of the warfarin enantiomers in the rat. J. Pharmacokin. Biopharm. 2: 239–255

Yacobi, A., Levy, G. (1977) Protein binding of warfarin enantiomers in serum of humans and rats. Ibid. 5: 123–131