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PHARMACOKINETICS OF THE ENANTIOMERS OF ACENOCOUMAROL IN MAN

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1 The pharmacokinetics of R(+)-, S(−)- and R,S(±)-acenocoumarol were studied in healthy volunteers after administration of single oral and intravenous doses.

2 After both oral and i.v. administration of either enantiomer in a dose of 0.25 mg/kg, the concentrations of R(+) found in the plasma were much higher than those of S(−). This indicates that the observed differences are not related to stereoselective absorption.

3 After intravenous administration of 25 mg of each enantiomer and the racemate, the total plasma clearance of S(−) was about 10 times that of R(+). The clearance of the racemate was between that of the enantiomers.

4 The apparent elimination half-life of S(−) was much shorter than those of R(+) and the racemate, which were similar.

5 The apparent volume of distribution V_dss of S(−) acenocoumarol was 1.5 to 2 times that of R(+).

6 Measurements of the extent of binding to serum proteins, made *in vitro* at much higher concentrations than those observed *in vivo*, revealed no differences between the two enantiomers and the racemate.

7 The results indicate that the greater anticoagulant potency of R(+) compared with S(−) acenocoumarol can be explained mainly by stereoselective differences in their metabolic clearance.

Introduction

Acenocoumarol (Sintrom®) has been in use as an anticoagulant for more than 20 years. Like the other single-ring coumarin derivatives, warfarin and phenprocoumon, it contains an asymmetric carbon atom and thus exists as two enantiomers. Commercial acenocoumarol is a racemic mixture consisting of equal parts of R(+) and S(−) enantiomers. Clinical pharmacology studies with the racemate have recently been reported (Hirtz *et al.*, 1979).

Several authors have shown that the enantiomers of warfarin and phenprocoumon differ in their pharmacokinetic characteristics and metabolic fate, as well as in their anticoagulant activity in man (O'Reilly, 1971; Hewick & McEwen, 1973; Lewis *et al.*, 1973; Breckenridge *et al.*, 1974; O'Reilly, 1974; Levy, O'Reilly & Wingard, 1974; Lewis *et al.*, 1974; Sellers & Koch-Weser, 1975; Hewick & Shepherd, 1976; Jähnchen *et al.*, 1976; Kelly & O'Malley, 1979). The S(−) enantiomers of these two drugs are twice to five times as potent as the R(+) enantiomers.

We have recently reported that the opposite is true of acenocoumarol (Meinertz *et al.*, 1978a,b): the R(+) enantiomer is several times more potent than the S(−) enantiomer in both man and rat.

As the greater anticoagulant activity of R(+) compared with S(−) acenocoumarol could reflect stereoselective differences in affinity for the receptor sites or in pharmacokinetic properties, we investigated the pharmacokinetics of the two enantiomers in man after the administration of a single oral or i.v. dose.

Methods

Subjects

The substances were given to four healthy male volunteers of ages ranging from 24 to 33 years and body-weights ranging from 71 to 84 kg.

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Materials

Racemic acenocoumarol and the optical enantiomers were supplied by Ciba-Geigy, Basle. The optical rotation of the S(−) acenocoumarol was −225° (1% in 0.5 N sodium hydroxide) and that of the R(+) acenocoumarol +233° (1% in 0.5 N sodium hydroxide), as measured by Ciba-Geigy, Basle. Racemic acenocoumarol was an equal mixture of the two enantiomers.

Drug administration and sample collection

The investigation was carried out in two parts: in the first (Study 1), all four healthy male volunteers received 0.25 mg/kg of each enantiomer intravenously in a cross-over experiment. Two of them were also given the same single dose orally as the racemate R,S(±) and each of the two enantiomers R(+) and S(−). An interval of 1 or 2 weeks was allowed between the successive doses. Blood samples were taken before and 6, 12, 24, 36, 48, 60 and 72 h after administration. An explorative determination of prothrombin times was performed with each plasma sample.

In the second part (Study 2), two of the same four volunteers received successive single intravenous doses of 25 mg of acenocoumarol as the racemate and each of the two enantiomers at 3-week intervals. This dose was administered in 1 minute after an overnight fast. A light standardized breakfast was taken 2 h and a normal lunch 4 h post-dosing. Blood samples were withdrawn before administration and 5, 15, 30 and 45 min and 1, 2, 3, 4, 6, 8 and 12 h thereafter. Further samples were taken 18, 24, 33, 36 and 48 h after injection of the racemate and the R(+) isomer.

In all cases, blood was withdrawn by venepuncture into plastic tubes containing 1 ml of 0.1 M citrate buffer solution. Plasma was separated and stored at −20°C until analysis.

The subjects received no other medication before and during the studies.

Analytical method

The concentrations of acenocoumarol were determined by a double radioisotope derivative method (Le Roux & Richard, 1977). Its limit of sensitivity is 20 ng/ml using 1.5 ml samples. The determinations were made in duplicate.

Protein-binding measurements

Protein binding of the racemate and enantiomers of acenocoumarol was determined by equilibrium dialysis at a total concentration of 10 μg/ml. Serum (1 ml) was dialysed for 6 h at 37°C against 1 ml of pH 7.4 phosphate buffer containing either the ^{14}C-labelled racemate (20 μCi/mg) or each of the unlabelled enantiomers. For the analysis of the racemate, samples of 0.5 ml were mixed with 10 ml Instagel and the radioactivity was measured. The concentrations of the enantiomers were measured by means of a gas-chromatographic method developed in our laboratories (Sioufi & Pommier, unpublished observations).

Owing to the detection limit of the cold analytical method, it was not possible to determine the free concentrations of acenocoumarol in the actual plasma samples of the treated subjects with accuracy.

Pharmacokinetic analysis

The main objective of Study 1 was to investigate the anticoagulant activity of the enantiomers of acenocoumarol. These results have already been reported (Meinertz *et al.*, 1978a,b). A pharmacokinetic treatment of the experimental data was not performed, since blood sampling was not frequent enough for that purpose.

The experimental data from Study 2 (25 mg i.v.) were fitted by non-linear regression analysis employing a Wang 702 computer and a programme based on multi-exponential equations and a total sum of squares with deviations expressed in percent. The apparent elimination half-lives were calculated from the exponential term describing the terminal segment of the curve, and the various pharmacokinetic parameters were derived from the multi-exponential equations.

The 25 mg racemate dose can be considered equal to the simultaneous administration of 12.5 mg of each enantiomer. Calculated plasma concentrations of R(+) were obtained by subtracting the contribution of S(−) to the observed concentrations of the racemate. The calculated values were also fitted by non-linear regression analysis. Pharmacokinetic parameters were derived from the parameters of the equation.

Results

Study 1

The data on the anticoagulant activity of the enantiomers have already been reported (Meinertz *et al.*, 1978a).

After oral administration, the levels of the R(+) enantiomer reached in the plasma were higher than those of the racemate (Figure 1). The R(+)/R,S(±) ratios of the concentrations found 6 h after administration were 4.6 and 2.1 for subjects 1 and 2, respectively.

Only minute amounts of acenocoumarol were detected in the plasma after either oral (Figure 1) or

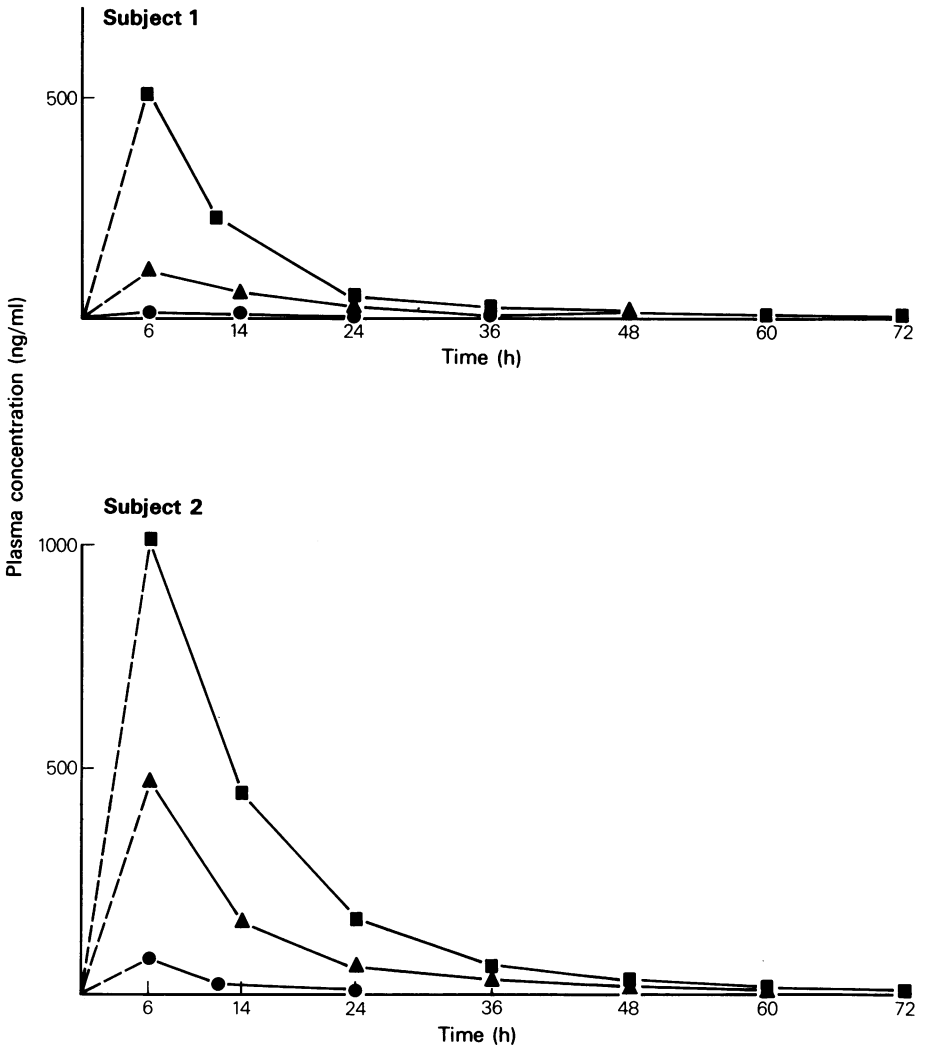

Figure 1 Plasma concentrations reached after oral administration of acenocoumarol racemate (▲) and enantiomers (S(−) ●, R(+) ■). Dose 0.25 mg/kg.

i.v. (Figure 2) administration of the S(−) enantiomer.

The similarity of the average areas under the plasma concentration-time curves for both routes of administration indicated that the availability of the R(+) enantiomer was complete. The availability of the S(−) enantiomer could not be quantified in this study, because of the very low plasma concentrations (Figures 1 and 2).

Study 2

The plasma concentrations of acenocoumarol reached after i.v. administration of 25 mg of the racemate and both enantiomers are shown in Figures 3 and 4. Non-linear regression analysis showed that the experimental data for the enantiomers R(+) and S(−) were best fitted by 3-exponential-term

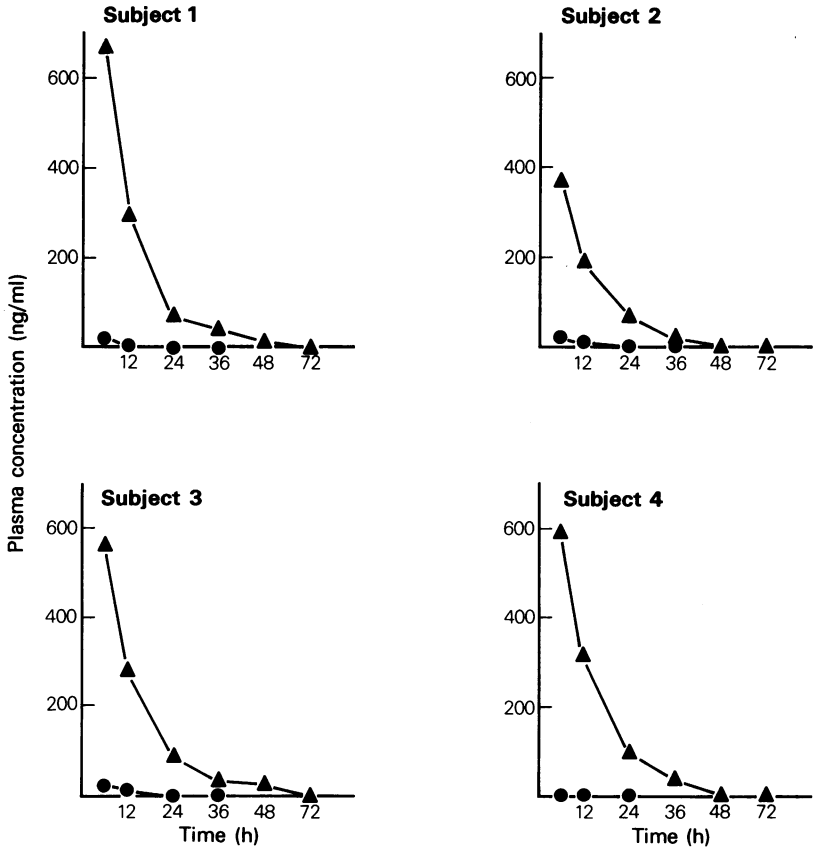

Figure 2 Plasma concentrations reached after i.v. administration of acenocoumarol enantiomers R(+) (▲) and S(−) (●). Dose 0.25 mg/kg.

equations. Since the availability of R(+) was similar after oral and intravenous administration, a three-compartment model not involving first-pass hepatic elimination had to be considered. The pharmacokinetic parameters associated with two such models (Figure 5) were calculated. The parameters common to both are summarized in Table 1.

The apparent elimination half-life ($T_{1/2}$) of the S(−) enantiomer was much shorter than those of the R(+) enantiomer and the racemate R,S(±). The total plasma clearance (Cl_T), the elimination rate (k_e), and the apparent volume of distribution (V_dss) were higher for the S(−) than for the R(+) enantiomer. The difference between the V_dss values does not seem to be related to differences in the apparent volume of distribution (V_c) of the central compartment.

Protein binding

The extent of protein binding of acenocoumarol determined at a total concentration of 10 μg/ml was found to be similar for both enantiomers and the racemate: 98.2 ± 0.18% for R(+), 98.0 ± 0.24% for S(−) and 97.9 ± 0.05% for R,S(±) (mean value ± s.d. of five determinations).

Discussion

R(+)- and S(−)-acenocoumarol differ in their pharmacodynamic properties: R(+) acenocoumarol is several times more potent as an anticoagulant than S(−) (Meinertz *et al.*, 1978a,b). These results are the

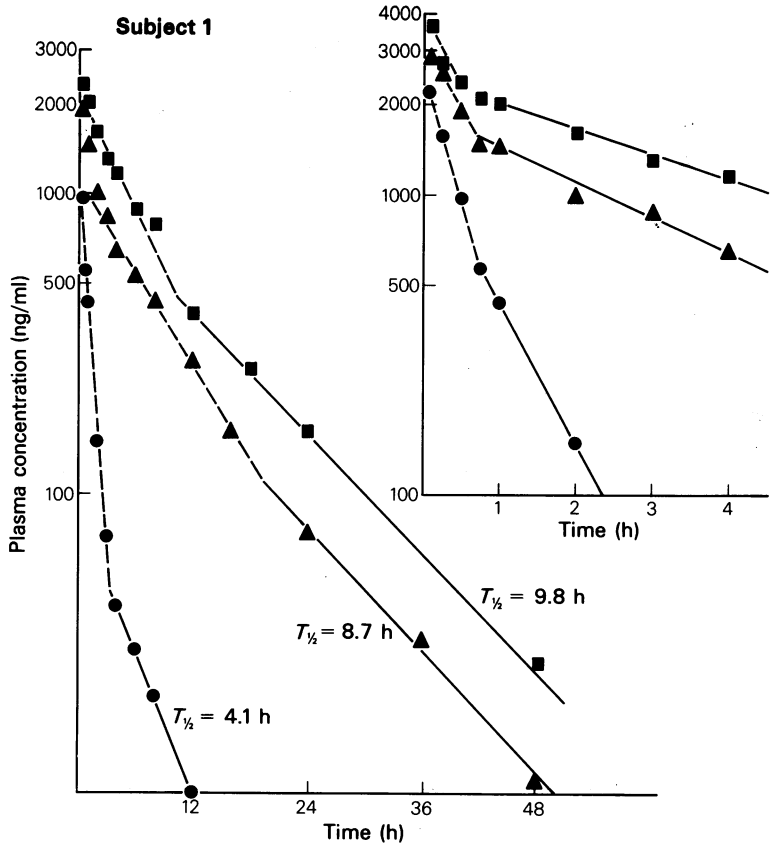

Figure 3 Plasma concentrations reached after i.v. administration of 25 mg of acenocoumarol racemate and enantiomers to subject 1. R,S(±) ▲, R(+) ■, S(−) ●.

opposite of those obtained with phenprocoumon (Jähnchen *et al.*, 1976) and warfarin (Breckenridge *et al.*, 1974; O'Reilly, 1974), the S(−) enantiomers of which were found to be two to five times more potent than the R(+).

Wheeler & Trager (1979) recently demonstrated that the reversed stereoselectivity with respect to the anticoagulant potency observed by Meinertz *et al.* (1978a) was not due to a misassignment of the absolute configuration of the enantiomers of acenocoumarol. In the present studies, we examined the possibility that the greater anticoagulant activity of R(+) compared with S(−) acenocoumarol might be due to stereoselective differences in their pharmacokinetics.

Study 1

This study was designed mainly to determine the anticoagulant activity of the two enantiomers (results reported by Meinertz *et al.*, 1978a,b).

The difference observed in the plasma concentrations of the two enantiomers R(+) and S(−) could not be due to stereoselective differences in the rate of absorption, since identical results were obtained after both oral and intravenous administration of the S(−) enantiomer.

It could be explained by stereoselective differences in the elimination rate, but the sampling schedule adopted in this study did not allow a complete pharmacokinetic investigation of the acenocoumarol enantiomers.

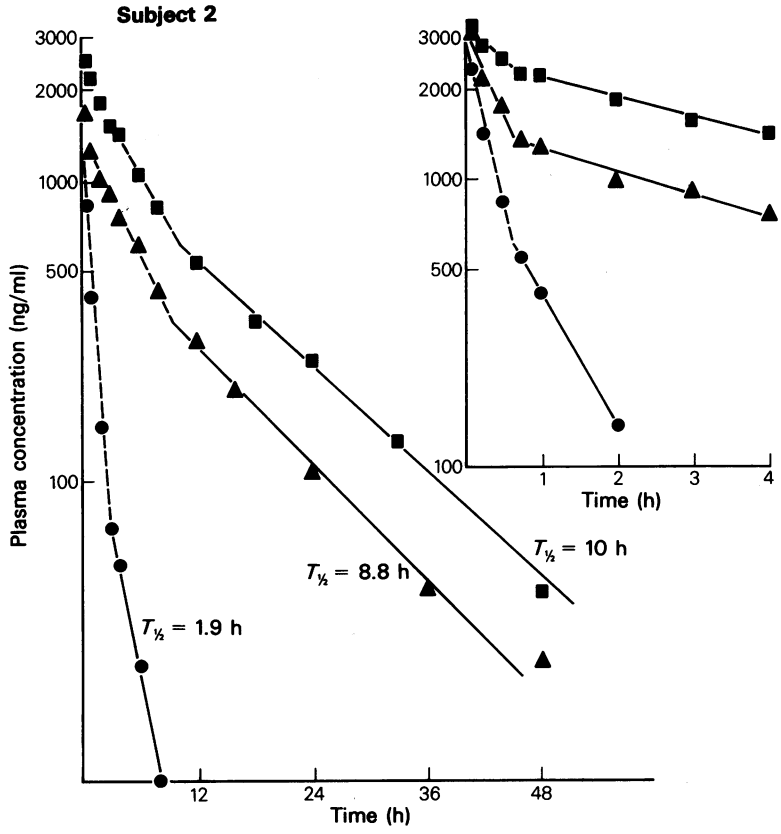

Figure 4 Plasma concentrations reached after i.v. administration of 25 mg of acenocoumarol racemate and enantiomers to subject 2. R,S(±) ▲, R(+) ■, S(−) ●.

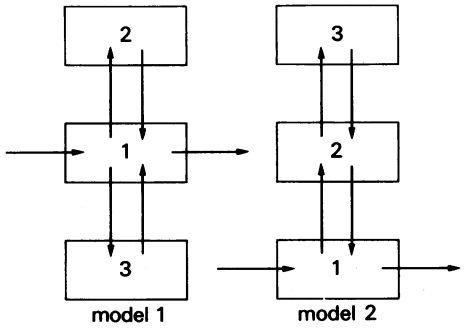

Figure 5 Three-compartment open models used to describe the kinetics of R(+) and S(−) acenocoumarol after intravenous administration of 25 mg of each enantiomer.

Since the availability of the R(+) isomer was similar after oral and intravenous administration, the anticoagulant potency of this substance was not reduced by a first-pass hepatic metabolism when given orally.

Study 2

After intravenous administration of 25 mg, the R(+) isomer yielded higher plasma levels of acenocoumarol in both subjects than did the S(−) isomer (Figures 3 and 4). Consequently, the area under the plasma concentration-time curve was larger for R(+) than for S(−) (Table 1).

The more potent R(+) acenocoumarol is eliminated more slowly than S(−) acenocoumarol: the elimination rate (k_e) and the total clearance (Cl_T) of the S(−) isomer were about 10 times higher than those of the R(+) isomer. Since acenocoumarol is eliminated almost entirely by hepatic metabolism (Dieterle *et al.*, 1977), the total plasma clearance

Table 1 Pharmacokinetic parameters of acenocoumarol enantiomers R(+) and S(−) and racemate R,S(±) common to the two models shown in Figure 5.

	Subject 1			*Subject 2*		
Parameter	R(+)	S(−)	R,S(±)	R(+)	S(−)	R,S(±)
$T_{½}$ (h)	9.8	4.1	8.7	10.0	1.9	8.8
AUC (μg ml^{-1} h)	18.2	1.8	11.1	22.4	1.6	12.5
Cl_T (l/h)	1.4	14.0	2.2	1.1	15.1	2.0
k_e (h^{-1})	0.24	1.64		0.16	2.22	
V_c (l)	5.7	8.6		6.8	6.9	
$V_d$ss (l)	14.4	27.4		13.4	18.9	

$T_{½}$, terminal elimination half-life; AUC, area under the plasma concentration-time curve; Cl_T, total plasma clearance; k_e, microscopic elimination rate constant; V_c, apparent volume of distribution of the central compartment; $V_d$ss, steady-state apparent volume of distribution.

mainly reflects the metabolic clearance of this drug. The apparent elimination half-life ($T_{½}$) of the racemate (Table 1) is similar to the values already reported after oral administration of 12 mg of ^{14}C-labelled racemate: 8.7 and 8.2 h for the two subjects (Dieterle *et al.*, 1977).

The apparent volumes of distribution of the two enantiomers differed by a factor of 1.5 to 2 (Table 1). A stereoselective difference in binding to serum proteins, which was reported to account for the differences in the volumes of distribution of phenprocoumon enantiomers (Jähnchen *et al.*, 1976), was not observed in the case of acenocoumarol enantiomers. However, owing to the limited sensitivity of the analytical method, the extent of binding was measured at concentrations much higher (10 μg/ml) than those observed in the subjects' plasma. Stereoselective binding at the high-affinity binding site(s) may have been masked by saturation due to the high drug concentration (Brown *et al.*, 1977).

Two other coumarinic anticoagulant drugs, phenprocoumon and warfarin, have been already reported to display such differences in the pharmacokinetic parameters of the enantiomers. Whereas the apparent volume of distribution of both warfarin enantiomers was found to be similar (Breckenridge *et al.*, 1974; Hewick & McEwen, 1973; O'Reilly, 1974), small differences have been described in the plasma protein binding of S(−) and R(+) warfarin (Kelly & O'Malley, 1979; O'Reilly, 1971; Sellers & Koch-Weser, 1975). Distinct differences were observed in the elimination rate, the less potent R(+) warfarin being eliminated more slowly than S(−) warfarin. These differences seem to result from different pathways of metabolism (Lewis *et al.*, 1974).

No difference has been observed in the rates of elimination of R(+) and S(−) phenprocoumon, but the apparent volume of distribution and the plasma clearance of the S(−) enantiomer were smaller than those of the R(+) enantiomer (Jähnchen *et al.*, 1976). The more potent S(−) was bound to a greater extent than R(+).

It is likely that the differences in the pharmacokinetic properties of acenocoumarol enantiomers, as well as those observed in their anticoagulant activity, mainly reflect differences in their metabolic clearance.

Acenocoumarol is extensively metabolized in man (Dieterle *et al.*, 1977). Reduction of the aromatic nitro-group yields the amino-metabolite, the major portion of which is further transformed to the corresponding *N*-acetyl metabolite. These two metabolites are the major biotransformation products in plasma. Reduction of the ketone-group yields two diastereoisomeric alcohol-metabolites, but it plays a subordinate role in the biotransformation of acenocoumarol. Oxidation of the coumarin nucleus results in the 6- and 7-hydroxy-metabolites.

The 7-hydroxy-metabolite proved to be inactive in mice, but the other metabolites induced a greater and longer-lasting depression of the prothrombin level than acenocoumarol (Dieterle *et al.*, 1977).

Whether the enantiomers of acenocoumarol undergo a stereoselective oxidative or reductive metabolism (as do the enantiomers of warfarin, Lewis *et al.*, 1974) has still to be determined. However, the much more potent anticoagulant activity of the R(+) enantiomer could be related to the formation of active metabolites predominantly from R(+) acenocoumarol.

The pharmacokinetic analysis of the data for the two enantiomers, based on three-compartment open models, suggested that the observed differences could also be explained by stereoselective differences in their distribution: the volume of the third compartment and the constants of distribution into this compartment were larger for S(−) than for R(+).

The kinetic and functional differences between the enantiomers of acenocoumarol emphasize that these compounds are quite dissimilar. As Breckenridge *et*

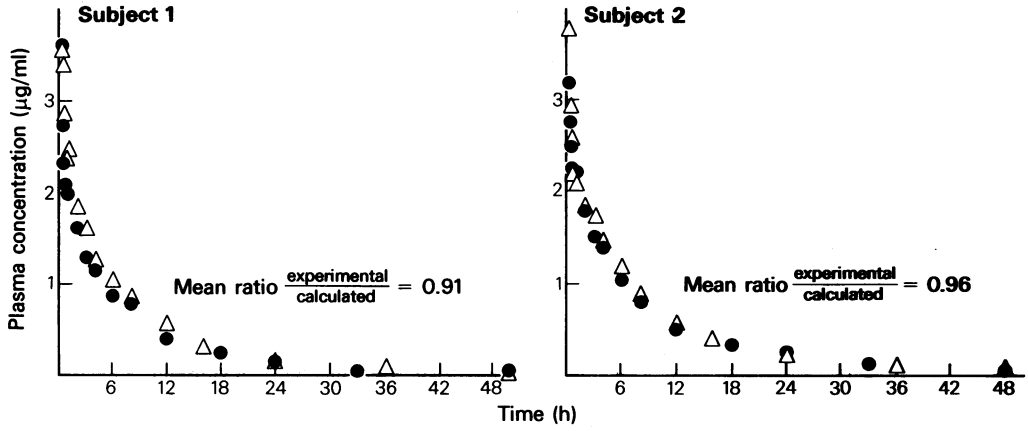

Figure 6 Experimental (●) and calculated (△) plasma concentrations of acenocoumarol corresponding to an i.v. dose of 25 mg of the R(+) enantiomer.

al. (1974) already remarked with regard to warfarin, they suggest that when the racemate of acenocoumarol is given 'one is administering not one but two drugs'. For this reason, conclusions concerning the racemate drawn on the basis of a pharamcokinetic model can be misleading.

The calculated plasma concentrations of R(+) closely agreed with those found experimentally (Figure 6): the mean ratios of the experimental to calculated concentrations at each sampling time were 0.91 and 0.96 for Subjects 1 and 2, respectively.

The pharmacokinetic parameters of the R(+) enantiomer determined from these concentrations (see pharmacokinetic analysis) were in agreement with those obtained from the experimental data. This demonstrates that there is no pronounced pharmacokinetic interaction between the two enantiomers after administration of the racemate in a single dose. However, as indicated by Levy, O'Reilly & Wingard (1978) in their paper on warfarin, these results do not preclude the occurrence of a significant interaction between the enantiomers of acenocourmarol during chronic administration of the racemate.

In conclusion, the greater anticoagulant activity of R(+) acenocoumarol can be explained mainly by its much lower plasma clearance. However, other factors, such as stereoselective differences in the intrinsic anticoagulant potency, drug distribution of stereoselective formation of active metabolites, may also be involved.

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