J. Pharm. Pharmacol. 1983, 35: 793–798
Received February 14, 1983

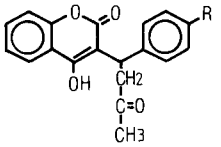
Acenocoumarol and its amino and acetamido metabolites. Comparative pharmacokinetics and pharmacodynamics in the rat

H. H. W. THIJSSEN*, L. G. BAARS, AND M. J. REIJNDERS

Department of Pharmacology, Faculty of Medicine, University of Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands

The elimination, distribution and anticoagulant activity of racemic acenocoumarol, its amino (AM) and acetamido (AA) derivative were determined in male Wistar rats after subcutaneous injection of a single dose of 2 mg kg^{-1}. The effect of daily administration of acenocoumarol on the prothrombin complex activity (PCA) was also investigated. A rapid onset of the hypothrombogenic effect was observed for all three derivatives with the half-life of decline of PCA=3·6 h. The duration of the hypothrombogenic effect was short for the drug and its AA derivative and long for the AM compound (normalization at about 24 and 70 h, respectively), parallelling the half-life of elimination of the compounds of, 3·3, 4, and 8 h respectively. Daily administration of acenocoumarol for 8 days showed no change in the kinetics of the anticoagulant effect. Elimination of the drug is solely by metabolism. Reduction of the 4′-nitro group was not a metabolic route of importance; the amount of its two derivatives cumulatively excreted in urine over 5 days accounted for 2–3% of the dose only. Elimination of AM derivative is mainly by acetylation to AA, the compound which itself is eliminated by renal excretion. The distribution of acenocoumarol between liver and plasma was determined. The liver to plasma ratio was higher than 1 beyond 10 min after administration. The elimination rate of the drug from liver was slower than from plasma giving an increase in liver to plasma ratio in time. Plasma protein binding was extensive, being the highest for the drug; 1·81, 2·84 and 3·89% free for drug, AM, and AA derivative, respectively.

Acenocoumarol is one of the 4-hydroxycoumarin congeners that are clinically in use as oral anticoagulants. Chemically the compound is different from warfarin by its 4′-nitro group (Fig. 1). Recently, Dieterle et al (1977), by using [^{14}C]-labelled acenocoumarol, showed in man the formation of the amino (AM) and acetamido metabolite (AA). As these metabolites were more potent than the drug in mice (Dieterle et al 1977), it was assumed that their formation in man might be responsible for the prolonged hypothrombinaemic effect of a once daily maintenance dose of drug (O'Reilly 1980).



R = NO_2	acenocoumarol
R = NH_2	amino
R = $NHCOCH_3$	acetamido
R = H	warfarin

FIG. 1. The structures of acenocoumarol, the amino (AM) and acetamido (AA) derivatives and of warfarin.

However, as the pharmacodynamic responses of the drug and its metabolites in mice were tested at relatively high doses (100 mg kg^{-1} for a single dose and 10 mg kg^{-1} for repeated doses; Dieterle et al 1977), and as the differences in hypothrombinaemic effects may be a reflection of pharmacokinetic rather than pharmacodynamic phenomena, a more detailed investigation of the pharmacokinetics and pharmacodynamics of these compounds has been undertaken and their disposition in racemic form and their effects on plasma thrombin time in rats is now described.

* Correspondence.

METHODS

Materials

[^{14}C]-Isotope labelled (±)-acenocoumarol (specific activity 10 μCi mg^{-1}) was a gift from Ciba-Geigy, Basel, Switzerland. Unlabelled drug was a gift from Ciba-Geigy, Arnhem, The Netherlands. The derivative AM was prepared from the drug by catalytic (Pt) hydrogenation. The AA derivative was prepared from the amino derivative by acetic anhydride treatment. The identity of the reaction products, AM and AA, was verified by comparison with authentic material obtained from Ciba-Geigy, Basel, Switzerland. (±)-[^{14}C] Warfarin (specific activity 49 μCi $mmol^{-1}$) was obtained from The Radiochemical Centre Amersham. Male inbred Wistar rats (Centraal Proefdierbedrijf TNO, Zeist, The Netherlands) weighing 270–320 g were used. The animals had free access to water and food.

Animal experiments

Three to four days before the experiment, rats were cannulated in the left femoral artery with a PE-50 catheter under slight ether anaesthesia. The tip of the catheter pointed upstream and reached to 5 mm distal from the left renal artery. The catheter was exteriorized in the neck and was flushed with 100 μl of heparin in 0·9% NaCl (saline) (50 u ml^{-1}). Following recovery the rats were acclimated to handling in metabolic cages.

Administration and sampling

Solutions of [^{14}C]-labelled drug and derivatives were prepared in 0·02M NaOH and stored at −20 °C. For administration, 4 × 10^6 d min^{-1} of the respective compound was mixed with an aliquot of a 2 mg ml^{-1} solution of unlabelled compound in 0·1M phosphate buffer pH 7·4, to a dose of 2 mg kg^{-1}. Administration was subcutaneously in the neck.

Blood samples were drawn from the catheter, 200 μl for drug analysis, and 100 μl for thrombotime measurements. The blood samples were transferred immediately into Eppendorf conical reaction vessels containing one tenth of the volume of 0·1 M trisodium citrate. The samples for prothrombin time assays were centrifuged in a Beckman microfuge at full speed, and plasma was drawn of and stored at −20 °C until assayed. The blood samples for drug analysis were diluted 1 : 1 with distilled water and stored at −20 °C until assayed. Following sampling, the catheter was filled with 50 μl of heparin in saline (50 u ml^{-1}). When a total of 1 ml of blood was withdrawn from the rat, an equivalent amount of blood, obtained from a donor rat, was given back. In this way, essential changes in blood and plasma composition (Hulse et al 1981) were prevented. For instance, by handling this way, the blood haematocrit decreased from about 46 to about 41% only, instead of to 36% or even lower (own results). Urine and faeces were collected for five days.

Fourteen days after the kinetic and dynamic experiment, the rats were used for the investigation of the distribution of drug over plasma and liver. At a time following the s.c. administration of drug (2 mg kg^{-1}), under ether anaesthesia the thorax was opened and blood was drawn from the vena cava as completely as possible in heparinized syringes, whereafter the liver was excised. Plasma was prepared immediately and divided in portions for drug assay and protein binding measurements. Liver homogenates were prepared in 1 to 1 dilution (w/v) in saline.

Drug analysis

The diluted blood samples were transferred to screw capped extraction vessels containing unlabelled drug, AA and warfarin (5 μg of each), 10 000 dpm of [^{14}C]-labelled warfarin, and 400 μl of a citric acid-phosphate buffer pH 4·4. Extraction was performed twice with 3·5 ml of dichloromethane–n-pentane (1 : 1). To separate the AM derivative from the other compounds, the combined organic phases were extracted with 0·4 ml of 0·5 M hydrochloric acid. The resulting organic phase was evaporated to dryness at 35 °C under a stream of nitrogen and the residue was taken up in 50 μl of chloroform.

Separation of the respective compounds was performed by thin-layer chromatography on silica gel (silica gel plates F254 with concentration zone, Merck, Darmstadt, GFR). The developing solvent was a mixture of toluene–ethylacetate–ethanol–acetic acid (25 : 25 : 2 : 0·2, v/v/v/v), giving R_F-values of 0·11, 0·50, 0·61, for AA, drug and warfarin, respectively. An aliquot of 25 μl of the final chloroform solution was spotted. Following chromatography, the spots were scraped off and transferred into scintillation vials containing 0.5 ml of 0·1% acetic acid in methanol. Pico-Fluor 15 (Packard, Bruxelles, Belgium) was used as scintillation liquid. The AM-phase, i.e. the hydrochloric acid phase, was counted in total. [^{14}C]Warfarin served as internal standard for the whole procedure. The recovery of the assay was: 91·4 (2·4), 83 (1), 88 (3), 87 (3·3)% (mean ± s.d., n=5) for drug, AM, AA derivatives and warfarin, respectively. Faeces were air dried, pulverized and extracted with hot methanol.

Liver and urine samples were analysed in the same way. For the analysis of conjugated products in urine, 0.5 ml of urine were incubated with 0·5 ml of a β-glucuronidase solution (β-glucuronidase, type VIII in 50% glycerol, Sigma) at pH 7 and 37 °C for 20 h.

Protein binding

Plasma protein binding was determined by equilibrium dialyses at 37 °C using the dianorm dialysis apparatus (Diachema AG, Switzerland). Estimations were performed in triplicate. Plasma (0·5 ml) was dialysed against an equal volume of 0·038 M NaCl in phosphate buffer, pH = 7·4, for 5 h.

Prothrombin time

The one-stage prothrombin time was converted to prothrombin complex activity (PCA) from a reference curve as described by Wingard & Levy (1973).

Pharmacokinetic analysis

The half-life of elimination was obtained from the slope of the β-phase. As the bioavailability of a subcutaneously applied dose was considered to be 100%, total clearance (TC) and apparent volume of distribution (Vd_{area}) were obtained by the area under the curve (AUC) method: TC=D/AUC, Vd_{area}=TC/β. AUC was estimated by the trapezoidal rule with the addition of Ct/β to extrapolate from the last sample (Ct) to t=∞.

RESULTS

The disposition of the drug in rats was investigated in two ways, (1) with the cannulated rat thereby allowing the investigation of the kinetics and dynamics in one individual, (2) using individual rats for a single sample point to estimate the distribution of drug in plasma and liver with time. The results of the former experiments are comparative with the results obtained for the derivatives, and will be presented first.

The mean time course of the blood concentrations of drug and AM and AA derivatives after a single subcutaneous dose (2 mg kg^{-1}) of each is shown in Fig. 2. As is evident, the AM derivative showed the lowest and the drug the highest rate of elimination from the blood. The respective blood half-lives of drug, AM and AA derivatives were 3.3 (0.3), 8 (1.2), and 4 (0.6) h (mean ± s.d.). Absorption from the site of application was rapid, giving peak concentrations between 10 and 30 min. Following absorption, the blood concentration of drug clearly showed a biphasic course with t½=0·7 h for the fast phase.

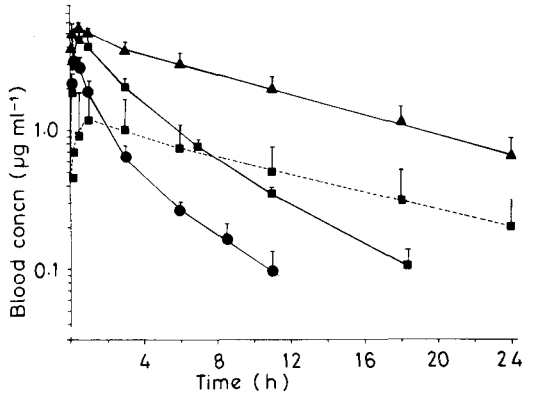


FIG. 2. The time course of the blood concentration (mean ± s.d.) of the respective coumarin derivatives following a single subcutaneous dose (2 mg kg^{-1}). A (n=5), ●——●; AM and its metabolite AA (n=4), ▲——▲, and ■- - - -■; AA (n=4), ■——■.

Following the administration of drug, no AM or AA derivative could be detected in the blood. Following the administration of the AM compound, its metabolite, the AA derivative, was found to be present within 5 min and to have its peak at about 1 h. Thereafter the AA compound declined in parallel with the AM derivative and constituted about 30% of the blood concentration of the latter. Only with the drug was another metabolite detected in the circulation. This was a compound migrating on t.l.c. with R_F = 0·36. This metabolite was present in amounts less than 10% of the drug.

Pharmacokinetic constants, plasma protein binding, and renal and faecal excretion, are given in Table 1. As is seen, the drug showed the highest total blood clearance and highest apparent volume of distribution (Vd_{area}). The three compounds showed extensive plasma protein binding, the drug being the most tightly bound.

The fraction of the dose excreted cumulatively over 5 days amounted to 40% (24% in urine) for the drug. 70% (65%) and 77% (67%), for the AM and AA derivatives respectively.

Table 1. Pharmacokinetic constants, protein binding, renal and faecal excretion of acenocoumarol (A) and its amino (AM) and acetamido (AA) derivatives in rats.

Parameter	A (5)[a]	AM (4)[a]	AA (4)[a]
t½ (h)	3·3±0·3	8±1·2	4±0·6
AUC (μg ml^{-1} min)[b]	477±59	3521±622	1115±152
AUC metab. (μg ml^{-1} min)[c]		900±490	
TC (ml h^{-1} kg^{-1})	254±31	35±6·6	106±17
Vd_{area} (ml kg^{-1})	1187±86	391±14	618±26
Free fraction × 100	1·81±0·49	2·84±0·26	3·89±1·1
Renal excretion (0–5 days):			
Total (% of dose)	24±4	65±6	67±3
Parent (% of dose)	1	11·5±3	65±4
Metabolite (% of of dose)	1,1·5[d]	45±9[e]	2·1±0·3[f]
Faecal excretion (0–5 days):			
Total (% of dose)	16±3	5±2	10±1–6

[a] Mean ± s.d.; n is the number in parentheses.
[b] Values are based on blood concentrations.
[c] The metabolite of AM is AA.
[d] The metabolites are AM and AA, respectively.
[e] The metabolite is AA.
[f] The metabolite is AM.

Fractionation of the acecoumarol-urine showed drug and AM and AA derivatives to be present in minor quantities only, 1 to 1·5% of the dose. Three other metabolites were found to be present, R_F = 0·24, 0·31, and 0·36, the latter being about 3% of the dose. Most of the radioactivity present in urine was not extractable (about 70%). Treatment of the urine with β-glucuronidase almost doubled the extractable radioactivity, which was mainly the result of a rise in the amount of the three undefined metabolites. No unchanged drug was found in faeces.

Analysis of the AM-urine showed that 11% of the dose was excreted as the AM and 45% as the AA derivative. The radioactivity recovered in urine after the administration of the AA derivative was mainly the unchanged compound.

The mean time course of PCA following the acute subcutaneous administration of the substances (2 mg kg^{-1}) is shown in Fig. 3. The same initial fall in PCA ($t_{1/2}$=3·6 ± 0·7 H; n=13) was observed for the three coumarin derivatives. For drug and AA derivative, the minimum in PCA (10% of normal) was observed at 12 h. At 24 h, PCA had returned to normal. For AM derivative, PCA had reached its minimum (2–3% of normal) at about 24 h and had returned to normal at 70 h. For comparison, the anticoagulant effect of the compounds was quantitated as the area under the effect (% PCA deficiency) – time curve (Jähnchen et al 1976), giving 1500 (145), 4750 (1450), and 1525 (110) % PCA deficiency h (mean ± s.d.), for drug, AM and AA derivatives, respectively.

The fluctuation in PCA following the daily administration of drug (2 mg kg^{-1}), either subcutaneously or intraperitoneally, is depicted in Fig. 4. As is seen 24 h after each administration, PCA had returned to about normal again.

The distribution of drug in plasma and liver with time following a single subcutaneous dose is shown in Fig. 5. Plasma as well as liver concentrations followed a biphasic course with half-lives for the fast and slow phases of 0·9 and 3·6 h for plasma, and 1·0 and 6·7 h for liver, respectively. Liver concentrations after 10 min were consistently higher than plasma concentrations and the liver/plasma ratio increased with time, e.g. a liver/plasma ratio of 4 at 8 h and of 13 at 24 h after the administration. At the T_{max} of the liver concentration, i.e. 30 min, 17% of the dose was present in the liver. At 8 h, this was about 2%, and at 24 h about 0·5%. The area under the plasma concentration time curve was 934 μg ml^{-1} min, and plasma clearance and Vd_{area} were 128 ml h^{-1} kg^{-1}, and 698 ml kg^{-1}, respectively. The AM metabolite

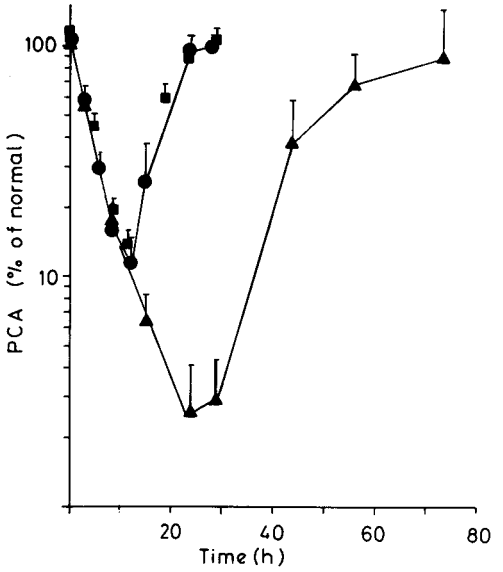


FIG. 3. The time course of the PCA (mean ± s.d.) following a single subcutaneous dose (2 mg kg^{-1}) of: A (n=5), ●——●: AM (n=4), ▲——▲; AA (n=4), ■——■.

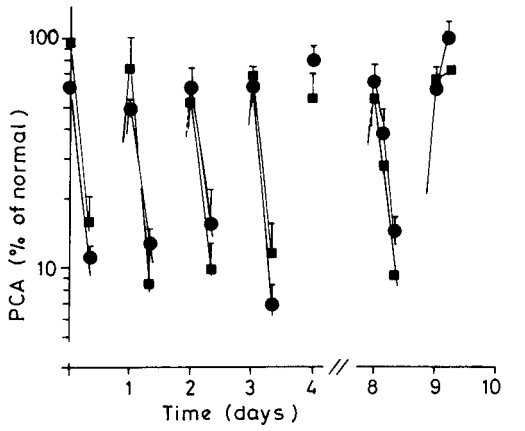


FIG. 4. The time course of PCA (mean ± s.d.) following the daily subcutaneous (●——●) or, intraperitoneal (■——■) administration of A (2 mg kg^{-1}) for eight consecutive days (n=3). N.B. One rat of the intraperitoneal series died on day 8 from haemorrhages.

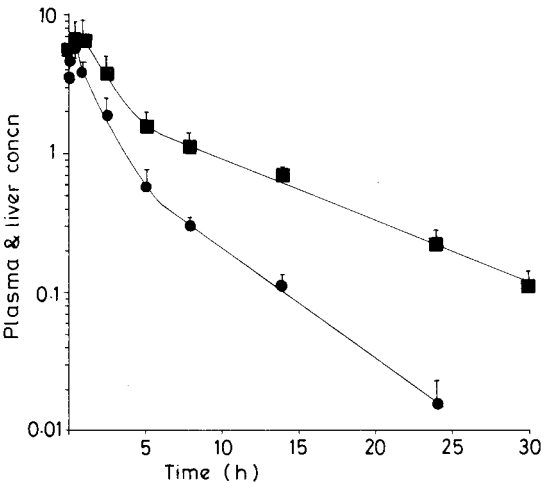


FIG. 5. The time course of the plasma and liver concentration of A following a single subcutaneous dose of 2 mg kg^{-1}. Each point represents the mean (± s.d.) of 4 rats. Plasma concentration (μg ml^{-1}), ●——●: liver concentration (μg g^{-1}), ■——■.

was present in plasma and liver in minor amounts, a maximum was observed at 8 h, 150 ng ml^{-1} in plasma and 160 ng g^{-1} in liver.

DISCUSSION

The 4-hydroxycoumarin derivative, acenocoumarol, is classified as a short-acting oral anticoagulant. Following a single dose of the racemic drug, prothrombin time usually returns to normal within 48–72 h in man (Blatrix et al 1968; Meinertz et al 1978), rabbit (Montigel & Pulver 1955), and mice (Dieterle et al 1977), and within 24 h in rat (Meinertz et al 1978; Thijssen et al 1982; this paper). This short duration of action, apparently is determined more by pharmacokinetic than by pharmacodynamic characteristics. In man, the plasma half-life of the racemic drug has been shown to be 3–9 h (Dieterle et al 1977; Houwert-de Jong et al 1981; Godbillon et al 1981), which is short when compared to the half-lives of warfarin ($t\frac{1}{2}$=42 h, O'Reilly et al 1963) or phenprocoumon ($t\frac{1}{2}$=about 6 days, Jähnchen et al 1976). An apparent half-life of elimination from plasma or blood of 3–4 h, as observed for racemic drug in male Wistar rats in this study, is short too when compared to a $t\frac{1}{2}$ of about 12 h for racemic warfarin in the same strain (own results). The derivative AA is rapidly eliminated too ($t\frac{1}{2}$=4 h), and the duration of the anticoagulation is comparable to that of the drug. The elimination of the AM compound is slower ($t\frac{1}{2}$=8 h), so the hypothrombinaemic effect is more pronounced and longer lasting.

The processes of elimination are different for the three compounds. The drug is almost completely metabolized. Although the metabolites in urine were not identified, their chromatographic behaviour and the results with deconjugation reactions make it likely that, by analogy with warfarin (Barker et al 1970), hydroxylation of the coumarin part of the molecule and subsequent conjugation is the main route of metabolism. Metabolism by a reductive pathway, i.e. reduction of the 4′-nitro group, hardly occurred following the s.c. administration. Intraperitoneal administration, as deduced from the time course of the pharmacodynamic effect (Fig. 4), did not lead to the formation of the AM compound in significant amounts. The minor contribution of aromatic nitro-reduction in the metabolism of the drug in rat is different from that seen in man (Dieterle et al 1977). As seems obvious now, the in-vivo reduction of aromatic nitro compounds is caused by intestinal flora rather than by liver systems (Wheeler et al 1975; Levin & Dent 1982), the above-mentioned discrepancy may be because the data in man were obtained after oral administration of the drug.

Excretion of the metabolic products of the drug is via urine and faeces, although only 40% of the dose was recovered in 5 days. This indicates that either the drug is stored in the body (see also later) or metabolites are formed with long half-lives of elimination. Expiration of $^{14}CO_2$ was not assessed. For warfarin, 14 days were needed to excrete 90% of the radioactivity of an i.p. dose. Excretion of the remaining radioactivity occurred over 90 days (Barker et al 1970). Elimination of the AM derivative is by acetylation to the AA compound which is mainly eliminated by renal excretion (65% of the dose). The differences in the kinetics of the AA compound when given per se and when formed from the AM derivative ($t\frac{1}{2}$=4 h vs. $t\frac{1}{2}$=8 h, Fig. 2), indicate that the formation of the AA compound was rate-limiting in its kinetics as a metabolite of the AM derivative.

The three compounds share the high affinity for plasma proteins (mainly to albumin) with other 4-hydroxycoumarin derivatives. The plasma protein binding of acenocoumarol and warfarin were about comparable in the rat strain used, 1·81 (±0·49) and 1·59 (±0·12)% free for drug (n=5) and warfarin (n=5), respectively. The weaker protein binding of the two derivatives may be due to their more polar properties (O'Reilly 1969).

The observed apparent volume of distribution of the drug (1187 or 698 ml kg^{-1}, for blood or plasma, respectively) is high when compared with warfarin (about 200 ml kg^{-1} for a free warfarin serum fraction of 1.8%, Yacobi & Levy 1975). This would indicate that notwithstanding the high protein bindings acenocoumarol distributes readily over tissues or accumulates somewhere. Data on its tissue distribution in rabbits, however, showed only liver and kidney concentrations to exceed plasma concentration (Montigel & Pulver 1955). In this study, the liver to plasma ratio was greater than one from 10 min after subcutaneous administration. For the drug, the liver half-life time of elimination was longer than the plasma half-life time of elimination ($P<0·001$, Fig. 5). Since it is reasonable to assume that elimination of the drug is solely via the liver, the results indicate the existence of either a kind of "irreversible" storage in the hepatocyte that maintains a concentration gradient for a flux from plasma to hepatocyte, or some carrier-mediated uptake system in the liver active enough to transport drug against a concentration gradient. The latter suggestion is supported by the results of Wosilait et al (1981) who showed the

uptake rate of warfarin by isolated hepatocytes to be saturable and to be higher than the rate of metabolism. Comparable in-vivo results in rats for warfarin were not found in literature. In guinea-pigs, the liver to blood ratio of radioactivity, 1 and 6 h after the i.p. administration of [^{14}C]warfarin was about 1 (Wong et al 1978). For phenprocoumon, after its intravenous injection liver and plasma concentrations declined in parallel (Schmidt & Jähnchen 1977).

The biphasic concentration-time course of the drug (Figs 2 and 3) is probably due to the kinetic differences of its optical isomers, *S*(−) and *R*(+). Godbillon et al (1981) recently showed in man the clearance of *S*(−)-drug to be about 10 times higher than the clearance of the *R*(+)-isomer. In rats too, the elimination of the *S*(−)-isomer was much higher (own results). So, the initial fast phase is mainly determined by the elimination of *S*(−)-isomer. By the time this has been eliminated (about 4 to 5 half-lives, i.e. about 4–5 h), the concentration time curve only represents the slow component *R*(+)-isomer ($t_{1/2}$=3·6 and 6·7 h, for plasma and liver, respectively).

Human and animal experiments have shown the *S*(−)-enantiomers of warfarin and phenprocoumon to be the most potent as anticoagulants (O'Reilly 1974; Lewis et al 1974; Jähnchen et al 1976; Schmidt & Jähnchen 1977; Wingard et al 1978). Also from estimates of the vitamin K_1 epoxide to vitamin K_1 ratios in plasma it was found that the *S*(−)-enantiomers of those coumarins inhibited the vitamin K_1 epoxide reductase system in the liver most effectively (Schmidt et al 1979). It is reasonable to assume that the *S*(−)-isomer of acenocoumarol is also the most active. However, because of its high clearance, its contribution to the hypothrombogenic effect is negligible (Meinertz et al 1978; Godbillon et al 1981).

Given the fact that 12 h after the administration of the drug, PCA rose quickly and returned to normal within 12 h, it has to be concluded that the inhibition of the synthesis of the vitamin K-dependent clotting factors ceased at the latest at 12 h. At that time, the liver concentration of the *R*(+)-isomer was about 750 ng g^{-1} (Fig. 5). For comparison, the minimal effective liver concentration of *R*(+)-warfarin, as calculated from the data of Yacobi & Levy (1974: Table 6) using a liver to plasma ratio of 2·4 for warfarin (Levy et al 1978), was 240 ± 140 ng g^{-1}. For *R*(+)-phenprocoumon, a minimal effective liver concentration of 1000 ng g^{-1} was calculated from relevant data (Schmidt & Jähnchen 1977).

The time courses of the hypothrombogenic effect following daily administration of acenocoumarol s.c. as well as i.p., for 8 days suggest that there are no gross changes in its pharmacokinetic or pharmacodynamic characteristics during its chronic administration.

REFERENCES

Barker, W. M., Hermodson, M. A., Link, K. P. (1970) J. Pharmacol. Exp. Ther. 171: 307–313

Blatrix, C., Choronnat, S., Tillement, J. P., Israel, J., Brevet, J. P., Debraux, J., Merlin, M. (1968) Rev. Fr. Etud. Clin. Biol. 13: 984–995

Dieterle, W., Faigle, J. W., Montigel, C., Sulc, M., Theobald, W. (1977) Eur. J. Clin. Pharmacol. 11: 367–375

Godbillon, J., Richard, J., Gerardin, A., Meinertz, T., Kasper, W., Jähnchen, E. (1981) Br. J. Clin. Pharmacol. 12: 621–629

Houwert-de Jong, M., Gerards, L. J., Tetteroo-Tempelman, C. A. M., De Wolff, F. A. (1981) Eur. J. Clin. Pharmacol. 21: 61–64

Hulse, M., Feldman, S., Bruckner, J. V. (1981) J. Pharmacol. Exp. Ther. 218: 416–420

Jähnchen, E., Meinertz, T., Gilfrich, H. J., Groth, U., Martini, A. (1976) Clin. Pharmacol. Ther. 20: 342–349

Levin, A. A., Dent, J. G. (1982) Drug Metab. Dispos. 10: 450–454

Levy, G., Lai, C. M., Yacobi, A. (1978) J. Pharmacol. Sci. 67: 229–231

Lewis, R. J., Trager, W. F., Chan, K. K., Breckenridge, A., Orme, M., Roland, M., Schary, W. (1974) J. Clin. Invest. 53: 1607–1617

Meinertz, T., Kasper, W., Kahl, C., Jähnchen, E. (1978) Br. J. Clin. Pharmacol. 5: 187–188

Montigel, C., Pulver, R. (1955) Schweiz. Med. Wschr. 24: 586–592

O'Reilly, R. A. (1969) J. Clin. Invest. 48: 193–202

O'Reilly, R. A. (1974) Clin. Pharmacol. Ther 16: 348–354

O'Reilly, R. A. (1980) in: Goodman Gilman, A., Goodman, L. S., Gilman, A. (eds) The Pharmacological basis of therapeutics. Macmillan Publishing Company, New York, p. 1359

O'Reilly, R. A., Aggeler, P. M., Leong, L. S. (1963) J. Clin. Invest. 42: 1542–1551

Schmidt, W., Beermann, D., Oesch, F., Jähnchen, E. (1979) J. Pharm. Pharmacol. 31: 490–491

Schmidt, W. Jähnchen, E. (1977) Ibid. 29: 260–271

Thijssen, H. H. W., Baars, L. G., Reijnders, M. J. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 319 (suppl.): 19

Wheeler, L. A., Soderberg, F. B., and Goldman, P. (1975) J. Pharmacol. Exp. Ther. 194: 135–144

Wingard, L. B., Levy, G. (1973) Ibid. 184: 253–260

Wingard, L. B., O'Reilly, R. A., Levy, G. (1978) Clin. Pharmacol. Ther. 23: 212–217

Wong, L. T., Solomonraj, G., Thomas, B. H. (1978) J. Pharm. Pharmacol. 30: 240–243

Wosilait, W. D., Ryan, M. P., Byington, K. H. (1981) Drug Metab. Dispos. 9: 80–84

Yacobi, A., Levy, G. (1974) J. Pharmacokin. Biopharm. 2: 239–255

Yacobi, A., Levy, G. (1975) J. Pharm. Sci. 64: 1660–1664