Biochemical Pharmacology, Vol. 37, No. 1, pp. 19–27, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

WARFARIN: METABOLISM AND MODE OF ACTION

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Abstract—The various stages involved in the transport, pharmacological action and elimination of warfarin involve the specific binding of warfarin to a chiral macromolecular complex. However, it seems that the degree of stereoselectivity is variable, which presumably reflects the importance of the side-chain in binding to each type of macromolecule. It would appear that there is greater stereoselective control in the interaction of warfarin with cytochrome P-450 enzymes than that observed for interaction with the receptor, vitamin K_1 epoxide reductase. Indeed, warfarin has been developed as a powerful stereochemical probe for *in vitro* studies of the terminal enzyme in the mixed-function oxidase system, cytochrome P-450. Warfarin undergoes hydroxylation in the 6, 7 and 8-positions of the aromatic ring which must interact with the active (haemoprotein) portion of the molecule, leaving the side-chain, which contains the chiral centre, free for recognition by the substrate binding site. *In vitro* studies indicate that the interaction of warfarin at its receptor, vitamin K_1 epoxide reductase, is completely non-stereoselective. This suggests that only the 4-hydroxycoumarin ring portion of the drug binds to the enzyme. Consistent with this hypothesis, salicylate, which can mimic part of the 4-hydroxycoumarin ring system, produces hypothrombinaemia by inhibition of vitamin K_1 epoxide reductase. These findings suggest that the coumarin ring system is largely responsible for the pharmacodynamic properties of warfarin, whereas the side-chain dictates the disposition and metabolism of the drug.

Thrombo-embolic disease is a major cause of mortality and morbidity [1]. Coumarin anticoagulants are widely used in the treatment of thrombo-embolic-disease and recently there has been renewed interest in the use of these drugs for the primary and secondary prevention of heart disease. Warfarin is the most commonly used coumarin in Britain and North America, while in mainland Europe both phenprocoumon and acenocoumarol are extensively used in addition to warfarin (Fig. 1). Dicoumarol is of interest for historical reasons, it was the original coumarin anticoagulant isolated by Campbell and Link [2] from spoiled sweet clover hay in investigations of the bleeding diathesis induced in cattle in North America in the 1930s. It was the first oral anticoagulant given to man, but is no longer used because of non-linear kinetics and poor absorption [3]. Brodifacoum is a potent rodenticide which acts like warfarin, but is equally effective in both warfarin-sensitive and warfarin-resistant rats [4, 5].

The essential chemical characteristics of the coumarin derivatives for anticoagulant activity are an intact 4-hydroxycoumarin ring with a carbon substituent. Warfarin, phenprocoumon and acenocoumarol contain an assymetrical carbon in the substituent at the three position, and the therapeutic preparations of the drugs are racemates. 4-Hydroxycoumarin anticoagulants act by inhibition of the vitamin K-dependent step in clotting factor synthesis. It is thought that coumarin anticoagulants inhibit the reduction of vitamin K 2,3-epoxide and vitamin K itself, in the physiologically important vitamin K-epoxide cycle [6, 7]. 4-Hydroxycoumarins have a high affinity for non-receptor macromolecules, such as plasma, tissue and hepatic proteins. In addition, these drugs undergo extensive metabolism which involves oxidation, reduction and glucuronylation.

Thus it can be seen that the pharmacological response to 4-hydroxycoumarins, such as warfarin may be regulated by a large number of pharmacodynamic and pharmacokinetic processes (Fig. 2). A major problem associated with the use of anticoagulants is the variation in pharmacological response in individuals. The aim of this review will be to consider the importance of stereochemistry in each of these processes and thus provide a clearer understanding of the use and limitation of the drug.

CHEMISTRY

Warfarin, and the related 4-hydroxycoumarin anticoagulants phenprocoumon and acenocoumarol possess a single asymmetric centre. The absolute configuration of warfarin is well established through the correlation of (*S*)-warfarin to (*S*)-(−)-β-phenylcaproic acid by a series of chemical transformations which do not involve the asymmetric centre [9], and by X-ray crystallography [10]. The absolute configuration of acenocoumarol has been assigned by chemical conversion of (−)-acenocoumarol to (*S*)-(−)-warfarin through a series of reactions not involving the asymmetric centre [10]. In solution, warfarin consists of three interconverting tautomeric structures (Fig. 3), two of which are cyclic diastereoisomeric hemiketals, while the third component is the open-chain intermediate form [11]. However,

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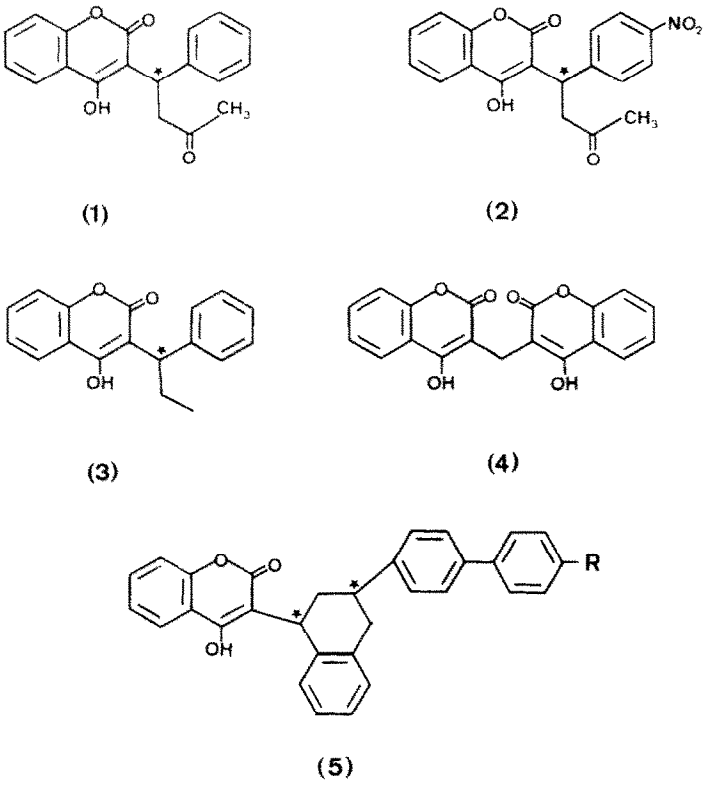

Fig. 1. Chemical structures of coumarin anticoagulants: (1) warfarin; (2) acenocoumarol; (3) phenprocoumon; (4) dicoumarol; (5) difenacoumon (R=H), brodifacoum (R=Br).

these data were obtained by X-ray crystallography and NMR spectra for Me_2SO-d_6 solutions, and extrapolations to the complexities of the microenvironment of biological systems, from pure solvents, should be made with caution. Nevertheless, each of the tautomeric forms must be considered in the interaction of warfarin with its receptor, and with those macromolecules involved in the disposition of the drug. Valente *et al.* [12] compared the conformations of 3-substituted 4-hydroxycoumarins in solution, by NMR, and suggested that the anti-vitamin K activity of warfarin is due to its open side-chain tautomeric form. Phenprocoumon and brodifacoum, which are more potent anticoagulants than warfarin, can only exist in an open-chain form [13, 14]. An octanol/water model system and circular

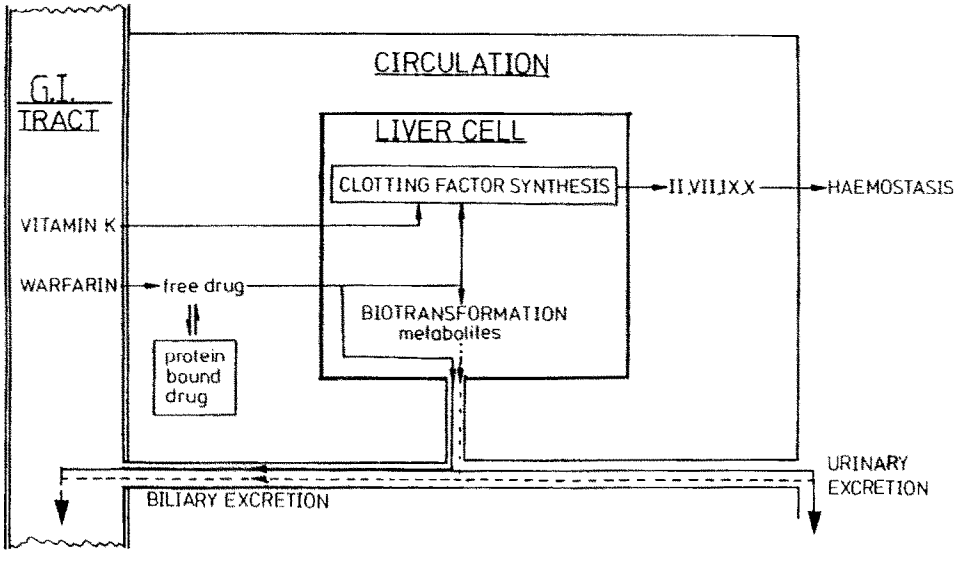

Fig. 2. Warfarin inhibits the vitamin K-dependent step in clotting factor synthesis.

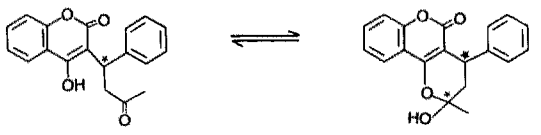

Fig. 3. Open-chain and cyclic hemi-ketal structures of warfarin in solution.

dichroism (CD) spectroscopy have been used to study the solution conformation of warfarin in aqueous and lipid environments [15]. Comparison of CD spectra of conformationally fixed analogues of warfarin to that of (*S*)-warfarin in solution suggests that the compound shifts from the open side-chain keto form in the aqueous phase at pH 7.4 to the cyclic hemiketal form after partitioning into the lipid octanol phase.

PHARMACOLOGICAL RESPONSE

The resolution of warfarin, by West *et al.* in 1961 made possible a study of the relative activity of the enantiomorphs [9]. It was found that a single dose of (*S*)-warfarin was 5.5 times as active as a single dose of *R*-warfarin in the rat prothrombin-time assay, as measured at 24 hr after dosing, while there was an 8.5-fold difference in LD_{50} determined at day 10 [16]. This difference is partly explained by a difference in the plasma half-life of the two enantiomers and partly by a difference in the inhibition of prothrombin complex synthesis; (*S*)-warfarin being two times more potent than *R*-warfarin in its ability to inhibit clotting factor synthesis [17].

In the rabbit, the duration of response to (*R*)-warfarin is longer than that to (*S*)-warfarin despite the fact that the latter is a more potent anticoagulant, with respect to the plasma concentration required for inhibition of clotting factor synthesis [18]. In man, the (*S*)-enantiomer has been reported to have 3.8 [19], 3.4 [20] and 2.7 [21] times the potency of (*R*)-warfarin. It has been shown in a number of studies that the clearance of the more potent enantiomer, (*S*)-warfarin, is greater than that for (*R*)-warfarin [22].

The (*R*)-enantiomer of (*R, S*)-acenocoumarol is several times more potent than the (*S*)-enantiomer. Examination of the pharmacokinetics of the enantiomers of acenocoumarol, after both oral and intravenous administration, revealed that the clearance of the (*R*)-enantiomer was approximately one tenth that of the *S*-isomer. Therefore, the greater anticoagulant activity of the *R*-isomer can be explained largely by its lower clearance, producing much higher plasma concentrations [23]. (*S*)-Acenocoumarol has been shown to be intrinsically more potent than its optical antipode in the rat [24].

Thus it can be seen that the overall pharmacological response to coumarin anticoagulants is dependent upon both pharmacokinetic and pharmacodynamic processes, which may have confounding stereoselective effects. In addition, there are apparent species differences in stereoselectivity. It is therefore necessary to explore the importance of stereochemistry in each process individually.

Coumarin derivatives reduce metastasis in experimentally induced tumours. However, it has not been established if this action is due to anticoagulation *per se*, or if there is a direct cytotoxic action on cancer cells and inhibition of cell motility and of mitotic activity [25, 26]. The effect of the two enantiomers of warfarin was investigated in mice bearing the Lewis lung carcinoma (3LL). Treatment of mice with warfarin was started 2 days before tumour-cell implant and continued until the animals were killed for histological investigation. When 3LL-cells were implanted intramuscularly, both primary tumour and spontaneous lung metastases were significantly reduced in mice treated with (*RS*)- or (*S*)-warfarin, but not in mice treated with (*R*)-warfarin [27]. The prothrombin test was consistently lower than 5% in animals given either racemic or (*S*)-warfarin. It ranged between 90% and 110% in both control animals and those which received (*R*)-warfarin.

ANTAGONISM OF VITAMIN K

Vitamin K is an essential cofactor for the post ribosomal synthesis of clotting factors II, VII, IX and X and the anticoagulant protein C [28]. The vitamin promotes the biosynthesis of γ-carboxyglutamic acid residues in the proteins which are essential for biological activity (Fig. 4). During the γ-carboxylation of glutamate residues, vitamin K_1 is converted into an inactive metabolite, vitamin K_1 epoxide, which is reduced back to vitamin K_1 and then to vitamin K_1 hydroquinone [7]. Warfarin and related coumarin anticoagulants are thought to interfere with clotting factor synthesis by inhibition of the regeneration of vitamin K_1 from vitamin K_1 epoxide [6]. Consistent with this hypothesis, it has been shown that administration of coumarin anticoagulants causes an accumulation of vitamin K_1 epoxide, in plasma, in man and experimental animals, after administration of either a physiological or pharmacological dose of the vitamin, and that the effect of warfarin on vitamin K_1 metabolism is dose-dependent [29–31].

The effect of the individual enantiomers of warfarin (1 mg/day) at steady state plasma concentrations has been investigated in volunteers. Both enantiomers produced a small but significant increase in prothrombin time [32] but the increase with (*S*)-warfarin was greater than with (*R*)-warfarin, despite lower steady state plasma concentrations of (*S*)-warfarin due to its more rapid clearance. Following the administration of vitamin K_1, the maximum plasma concentration and area under the plasma concentration time curve values for the metabolite, vitamin K_1 epoxide, were greater after (*S*)-warfarin than after (*R*)-warfarin. Thus the greater anticoagulant potency of (*S*)-warfarin is reflected by a greater degree of inhibition of vitamin K_1 epoxide reductase.

These *in vivo* observations are not in complete agreement with an *in vitro* study with rat liver microsomes [7]. The effect of the enantiomers of warfarin

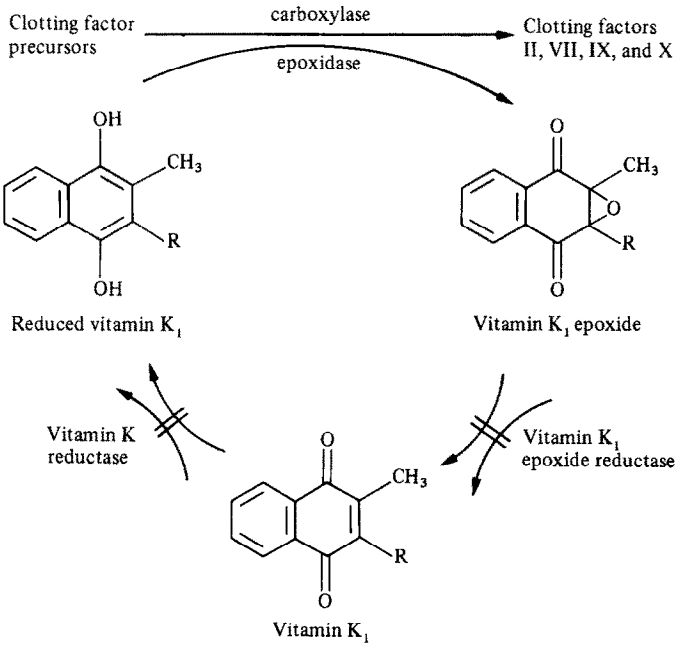

Fig. 4. The vitamin K_1-epoxide cycle and its role in clotting factor synthesis.

on vitamin K reductase and vitamin K epoxide reductase was studied *in vitro* with Wistar rat hepatic microsomes. (*R*) and (*S*)-warfarin were found to be equally effective as inhibitors of the reduction of both vitamin K_1 and vitamin K_1 2,3-epoxide, when added to microsomal systems. However, (*S*)-warfarin was a much better inhibitor of the two reductases *in vitro*, when administered *in vivo* at equivalent plasma concentrations to (*R*)-warfarin, prior to preparation of the microsomes. Why is there a difference in stereoselectivity between the *in vitro* and *in vivo* experiments? These results suggest that there is not a stereoselective interaction at the warfarin receptor, but that there is some stereoselectivity in the intrahepatic disposition/accumulation of warfarin. This may involve stereoselective metabolism and/or partitioning between membranes. To investigate this further, experiments carried out under conditions of steady-state hypothrombinaemia and warfarin plasma concentrations are essential. In this respect it is interesting to note that there is a slight difference in the liver:plasma concentration ratios of the enantiomers of phenprocoumon after administration to the rat. However, the difference is not great enough to account for the greater anticoagulant potency of *S*-phenoprocoumon [33].

Vitamin K_1 epoxide can exist as a pair of optical isomers due to the asymmetry of the oxirane ring substituents. Preusch and Suttie [34] investigated the stereoselectivity of vitamin K-epoxide reductase for the oxirane ring configuration with rat liver microsomes. Selectivity for the biologically relevant (+)-isomer was low, as was the preference of the reductase for the *cis* or *trans*-phytyl configuration of the phytyl side-chain. It was suggested, therefore, that the active site of the enzyme is open towards the 2,3-position and is able to bind the substrate in two opposite orientations with respect to the positions of the methyl and phytyl side-chains.

The lack of stereoselectivity in epoxide reduction contrasts with the formation of epoxide in the vitamin K-epoxide cycle. The coupled formation of vitamin K_1 epoxide by vitamin K-dependent carboxylase is highly stereospecific *in vitro* [34, 35].

Vitamin K_1 exists as a *cis* and a *trans* isomer because of the presence of a double bond in the 2',3' position of the lipophilic side-chain. The isomers were separated at a purity of better than 99.5% and tested for effect on plasma activity of factor VII in coumarin anticoagulant-pretreated and vitamin K-deficient rats [36]. Both isomers showed activity, but in coumarin anticoagulated animals the *cis* isomer has approximately 10% and in vitamin K-deficient approximately 1% of the activity of the *trans* isomer. We have investigated the pharmacological response to the *cis/trans* isomers of vitamin K_1 in rabbits chronically anticoagulated with the potent coumarin anticoagulant brodifacoum. The relative activity of the two isomers was dose-dependent and time-dependent. After intravenous administration of vitamin K_1 (1 mg/kg), prothrombin complex activity rose from $\leq 20\%$ to 64% within 3 hr, while equivalent doses of *cis*-vitamin K_1, vitamin K_2 and 2'3'-dihydro-vitamin K_1 were ineffective [37]. Interestingly, after administration of a higher dose (10 mg/kg) of *cis*-vitamin K_1 there was a delayed

pharmacological response. This response coincided with the appearance of *trans*-vitamin K_1 in plasma, which indicates that vitamin K_1 may undergo isomerization *in vivo* [38]. Taken collectively, these data suggest that there are precise structural requirements for the side-chain in vitamin K_1 during interaction with the epoxidase/carboxylase enzyme. In contrast, the side-chain does not play a major role in the recognition of vitamin K_1 epoxide by the epoxide reductase, which is thought to be the coumarin receptor.

ABSORPTION AND ELIMINATION

In general, it appears that drug absorption and renal excretion do not show significant stereoselectivity, apart from a small number of cases which involve active transport [39]. Warfarin is more than 95% absorbed after oral administration [40], the time to peak plasma concentration ranges from 0.5 to 4 hr. Both phenprocoumon and acenocoumarol are well and rapidly absorbed, the absorption of bishydroxycoumarin is much less complete and more erratic, which accounts for its infrequent use in man [41, 42]. There is no evidence for stereoselectivity in the rate of absorption of these drugs.

Warfarin, acenocoumarol and phenoprocoumon are extensively metabolised, predominantly in the liver and are excreted as metabolites which have little pharmacological activity. Renal clearance is therefore considered to be a minor determinant of the anticoagulant response to these drugs.

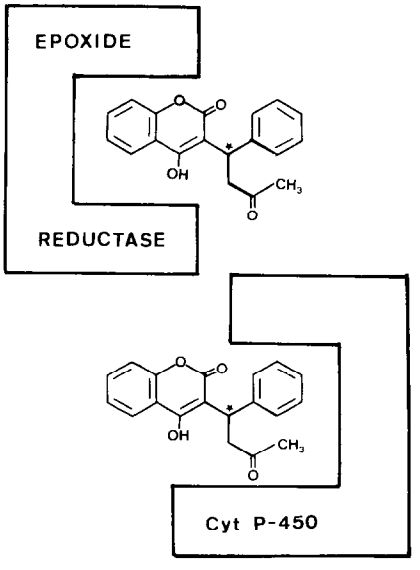

Fig. 5. Hypothetical scheme which depicts the recognition of warfarin by its receptor, vitamin K epoxide reductase, and the enzyme responsible for its metabolism, cytochrome P-450.

DISTRIBUTION AND PROTEIN BINDING

Warfarin has a relatively small volume of distribution of about 0.7 l/kg in man [22]. Using a one compartment model, and assuming complete bioavailability, estimates of the volume of distribution of (*R*)- and (*S*)-warfarin are similar to each other and to that of the racemate [19, 43, 44]. The clearance of (*R*)-warfarin is approximately half that of (*S*)-warfarin, thus as the volumes of distribution are similar, the half-life of (*R*)-warfarin is longer than that of (*S*)-warfarin.

Warfarin, phenprocoumon and acenocoumarin are all extensively bound ($\geq$ 98%) to serum albumin [45]. Most chiral drugs which are extensively bound by blood proteins, and in particular by serum albumin, will exhibit stereoselectivity. For both warfarin and phenprocoumon, the binding of the (*S*)-enantiomer is greater than for the (*R*)-enantiomer, although the difference is only of statistical significance for phenprocoumon [46, 47].

METABOLISM OF COUMARIN ANTICOAGULANTS

From a pharmacological point of view it is important to determine any stereoselectivity in factors which affect the rate of metabolism of the enantiomers of warfarin, and, in particular, the rate of clearance from the receptors. To achieve this, the routes of metabolism of the individual enantiomers must be defined. The metabolism of warfarin has been studied *in vitro* and *in vivo*. The drug can undergo numerous biotransformations including oxidation and reduction.

After administration of pseudoracemic warfarin to volunteers, an average of 50% of the dose was recovered in urine in the form of oxidative and reductive metabolites [48]. Of the 50% recovered, 19% arose from the (*R*)-enantiomer and 31% from (*S*)-enantiomer. The relative proportions of the metabolites are shown in Table 1; (*S*)-7-hydroxywarfarin was the major urinary excretory product. It is though that two distinct reductases are involved in the metabolism of (*R*)-warfarin.

The oxidation of warfarin by human hepatic cytochromes P-450 is complex, and the drug has in fact been used as a stereochemical probe for the multienzyme complex. Kaminsky and co-workers [49]

Table 1. The percentage of warfarin dose excreted as metabolites or as unchanged warfarin in man

	R	*S*
Warfarin	1.33 ± 0.55	1.05 ± 0.24
Alcohol 1	5.79 ± 0.89	0.06 ± 0.14
Alcohol 2	0.22 ± 0.14	1.95 ± 0.48
6-Hydroxywarfarin	8.91 ± 1.1	6.31 ± 0.50
7-Hydroxywarfarin	3.26 ± 1.69	21.71 ± 4.64
	19.06 ± 2.57	30.77 ± 4.66

Data taken from Toon *et al.* [48].

Table 2. Mean rates of formation of the metabolites from (*R*)- and (*S*)-warfarin catalyzed by hepatic microsomal preparations

Warfarin metabolite	Rate of metabolite formation	
	(*R*)-warfarin	(*S*)-warfarin
Dehydrowarfarin	22 ± 17	29 ± 24
4′-Hydroxywarfarin	17 ± 10	32 ± 16*
6-Hydroxywarfarin	111 ± 90	38 ± 20*
7-Hydroxywarfarin	44 ± 30	113 ± 73*
8-Hydroxywarfarin	46 ± 34	1 ± 3*
10-Hydroxywarfarin	40 ± 26	9 ± 8*

* $P \leq 0.05$. Mean ± SD (N = 33).
Data taken from Kaminsky *et al.* [51].

studied the *in vitro* metabolism of warfarin by human liver microsomal fractions from 27 renal donors and six cancer patients. Cytochrome P-450 enzymes catalyze the oxidation of warfarin to dehydrowarfarin and 4′-, 6-, 7-, 8- and 10-hydroxywarfarin. Overall, the microsomal preparations were stereoselective for *R*-warfarin metabolism. Of the 33 microsomal preparations, 21 exhibited qualitatively similar warfarin metabolite profiles, with 6*R* and 7*S*-hydroxywarfarin having the highest rates of formation (Table 2). It is interesting to note, however, that 8-hydroxylation of warfarin is the most stereoselective process.

The influence of conformation on the regioselective and stereoselective hydroxylation of warfarin and phenprocoumon by rat liver microsomes, has been considered in detail by Trager and coworkers. 3-Methylcholanthrene (3-MC) and β-naphthoflavone (BNF) have been found to induce form(s) of cytochrome P-450 which not only show a high affinity for warfarin and phenprocoumon, but also metabolise the two compounds with a high degree of stereoselectivity. 3-MC and BNF selectively induce formation of the 6- and 8-hydroxymetabolites; however, the stereoselectivity associated with these processes is for the opposite enantiomers of the two drugs, namely (*R*)-warfarin and (*S*) phenprocoumon [50, 51].

One possible explanation for this apparent paradox is provided by metabolic studies on conformationally restricted analogues of warfarin [52]. The (*R*)-enantiomer of the cyclic ketal analogue of warfarin was found to be selectively hydroxylated in the 6- and 8-positions. In contrast it was the (*S*)-enantiomer of warfarin 4-methyl ether, the ring-opened analogue of warfarin, which is selectively hydroxylated in these positions. It was suggested, therefore, that at the active site of BNF-induced cytochrome P-450 (*R*)-warfarin is metabolised as its cyclic hemiketal tautomer, a form which spatially mimics the preferred conformation of (*S*)-phenprocoumon [15].

Several groups have shown that in general the clearance of (*S*)-warfarin is greater than that of *R*-warfarin in man [22]. However, we have found that there is considerable variation between patients in the percentage of total warfarin in patient plasma which is present as (*S*)-warfarin.

DRUG INTERACTIONS

There have been a number of reports of stereoselective drug interactions with coumarin anticoagulants, most of which have been shown to have a pharmacokinetic basis. As stated above (*R*)-warfarin is less potent than (*S*)-warfarin. Nevertheless doses of (*R*)-warfarin as low as 1 mg daily do cause a measurable increase in prothrombin time in healthy volunteers [32]. It is therefore important to consider the implications of an interaction with either isomer. Enantioselectivity can be either quantitively or qualitatively discriminating with respect to metabolism; rate and route of metabolism of the individual isomers must be determined.

One of the most widely documented interactions of oral anticoagulants is with the anti-inflammatory drug phenylbutazone. Phenylbutazone may cause a marked potentiation of the hypothrombinaemic response to warfarin, which can be life-threatening [53]. Phenylbutazone potentiates the anticoagulant response to (*R*,*S*)-warfarin without any change in either clearance or plasma half-life of the racemate. The explanation for this clinically important interaction was provided by an investigation of the pharmacokinetics of the individual enantiomers of warfarin [43]. It was found that phenylbutazone increased the clearance of the less active (*R*)-warfarin, and decreased the plasma half-life. These effects are thought to be due to a displacement of the enantiomer from plasma and tissue binding sites, leaving more unbound (*R*)-warfarin available for elimination. In contrast, the clearance of the more active (*S*)-warfarin was reduced by phenylbutazone presumably by stereoselective inhibition of the metabolism of the isomer, which overrides any change in protein binding. The unbound clearance of (*S*)-warfarin is decreased fourfold during phenylbutazone administration due to substantial inhibition of both 6- and 7-hydroxylation.

The uricosuric and antiplatelet drug sulphinpyrazone markedly augments the hypothrombinaemic effect of warfarin when the two drugs are administered together. To allow simultaneous investigations of the interaction between sulphinpyrazone and the individual isomers of racemic warfarin, pseudoracemic warfarin (1:1 ^{12}C-*R*(+) and ^{13}C-*S*(−)warfarin) was given to volunteers, both before and during oral sulphinpyrazone dosing [48]. Blood and urine samples were analysed for unchanged warfarin, and its metabolites, by GC/MS. Concomitant administration of sulphinpyrazone decreased the clearance of (*S*)-warfarin, primarily by inhibition of the cytochrome P-450-mediated oxidation of (*S*)-warfarin. There was an increase in the clearance of (*R*)-warfarin which resulted from its selective displacement from plasma protein binding sites, rather than induction.

The bactericidal agents, mitronidazole and cotrimoxazole are thought to selectively inhibit the oxidation of (*S*)-warfarin while having no effect on (*R*)-warfarin. Thus, with each drug there was an increase in the mean plasma half-life of the (*S*)-enantiomer while that of (*R*)-warfarin remained unchanged [54, 55].

In contrast, we have found that interaction

between the H_2-antagonist cimetidine and warfarin involves a stereoselective interaction with (*R*)-warfarin. The warfarin enantiomers were given separately, as single doses alone and during chronic administration of cimetidine. Cimetidine did not interact with (*S*)-warfarin but did produce a significant increase in the mean plasma half-life of (*R*)-warfarin from 47.8 hr to 57.8 hr [56]. Studies with human liver microsomes *in vitro* [49] have shown that each enantiomer of warfarin may undergo at least six separate cytochrome P-450 mediated reactions. The major metabolites for (*R*)- and (*S*)-warfarin are the 6-hydroxy and the 7-hydroxywarfarin respectively. Thus, the stereoselective effect of cimetidine may in fact be due to a regioselective effect on 6-hydroxylation as opposed to 7-hydroxylation.

Not all interactions are stereoselective. The pharmacokinetic interaction between chloramphenicol and warfarin in the rat is due to non-stereoselective inhibition of metabolism [57]. However, the doses of inhibitor employed in this study were high.

At one time it was suggested that drug interactions with warfarin could be avoided by the clinical use of a single enantiomer. This early optimism is no longer warranted.

CONCLUSIONS

The various stages involved in the transport, pharmacological action and elimination of warfarin are outlined in Fig. 2. Each of these processes involves the specific binding of warfarin to a chiral macromolecular complex. However, it seems that the degree of stereoselectivity is variable, which presumably reflects the importance of the side-chain in binding to each type of macromolecule. It would appear that there is greater stereoselective control in the interaction of warfarin with cytochrome P-450 enzymes than that observed for interaction with the receptor, vitamin K_1 epoxide reductase. The importance of enantioselectivity in the metabolism of warfarin has been highlighted by the discovery of clinically important drug interactions which have a stereoselective basis. Indeed, warfarin has been developed as a powerful stereochemical probe for *in vitro*, and to a lesser extent *in vivo* studies of the terminal enzyme in the mixed-function oxidase system, cytochrome P-450. Warfarin undergoes hydroxylation in the 6, 7 and 8-positions of the aromatic ring which must interact with the active (haemoprotein) portion of the molecule, leaving the side-chain, which contains the chiral centre, free for recognition by the substrate binding site.

The pharmacological response to warfarin is less stereoselective than that observed for drugs such as isoprenaline, picenadol and ketamine for which there is considerable variation in both the potency and the properties of the enantiomers [58]. Indeed, *in vitro* studies indicate that the actual interaction of warfarin at its receptor, vitamin K_1 epoxide reductase, is completely non-stereoselective. This suggests that only the 4-hydroxycoumarin ring portion of the drug binds to the enzyme. Consistent with this hypothesis, we have shown that salicylate, which can mimic part of the 4-hydroxycoumarin ring system, in high doses produces hypothrombinaemia by inhibition of vitamin K_1 epoxide reductase [59]. It is interesting that the enzyme recognises the naphthoquinone nucleus of vitamin K_1 epoxide but not the substituents in the 2- and 3-positions. These findings suggest that the coumarin ring system is largely responsible for the pharmacodynamic properties of warfarin, whereas the side-chain dictates the disposition and metabolism of the drug (Fig. 5). Accordingly, we have shown that the extremely potent anticoagulant brodifacoum, which contains a large, lipophilic biphenyltetrahydronaphthyl side-chain has an extremely long half-life [60]. Other workers have shown that the marked difference in potency in man of the enantiomers of acenocoumarol, can be explained entirely by the rates of metabolism of the individual enantiomers.

Acknowledgements—I should like to thank Miss S. Pratt and Miss S. Oliphant for their help in the preparation of this manuscript.

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