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MECHANISM OF TICRYNAFEN POTENTIATION OF COUMARIN ANTICOAGULANT ACTION*

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Abstract—Administration of the antihypertensive drug ticrynafen [2,3-dichloro-4-(2-thienylcarbonyl)-phenoxyacetic acid] has been reported to potentiate the effects of coumarin anticoagulants and to have caused hemorrhagic incidents in some patients. This drug interaction has now been reproduced in the rat. Ticrynafen administration enhanced the degree of hypoprothrombinemia and altered plasma and hepatic vitamin K epoxide concentrations in warfarin-treated rats. Ticrynafen did not affect vitamin K-dependent carboxylase or vitamin K epoxide reductase activities *in vitro*. Cytosolic DT-diaphorase was very sensitive to ticrynafen inhibition *in vitro*, and inhibition of vitamin K reduction via this enzyme is a possible mechanism by which ticrynafen potentiates coumarin anticoagulant action. Inhibition of this enzyme may also contribute to the reported hepatotoxicity of ticrynafen.

Ticrynafen [2,3-dichloro-4-(2-thienylcarbonyl)-phenoxyacetic acid] is an antihypertensive agent with both natriuretic and uricosuric activities [1–3]. Routine clinical use of this drug resulted in reports that it caused hemorrhagic reactions by potentiating the intensity and duration of activity of several coumarin anticoagulants [4–8]. Coumarin anticoagulant therapy is often complicated by drug interactions, and most are thought to involve either the displacement of the anticoagulant from serum protein binding sites or interference with the rate of metabolism or clearance of the drug [9]. Limited data are available to explain the ticrynafen–coumarin interaction. Direct binding studies suggest that dissociation of warfarin from its serum binding sites by ticrynafen is not an important factor at normal serum protein concentrations [10, 11]. Elevated plasma warfarin levels in one study [8] suggest that ticrynafen may interfere with metabolism of the coumarin anticoagulants.

Subsequent to reports that ticrynafen was hepatotoxic [12–17], the drug was removed voluntarily from the market, at least in the United States. General hepatotoxicity does not appear to be the immediate cause of ticrynafen potentiation of coumarin anticoagulant action [8]. Though no longer of strong clinical interest, the mechanism of ticrynafen–coumarin interaction is of interest in the design of potential replacement drugs and as a probe of vitamin K and coumarin anticoagulant action. The reported potentiation of coumarin action in the human has now been observed in the rat. The sensitivities of several vitamin K-dependent rat liver enzymes to ticrynafen, and to warfarin alone and in the presence of ticrynafen, were examined and provide a basis for understanding the interaction between these two drugs.

MATERIALS AND METHODS

Except as noted, all procedures were conducted as previously described [18]. Male 250 g Holtzman strain rats were housed individually in wire-bottomed cages and provided commercial rat chow and drinking water *ad lib*. Ticrynafen was administered by gavage as a 100 mg/ml suspension in 10% sucrose or as a solution in ethanol. Control animals received identical treatments with vehicle alone. Additional treatments are given in the footnotes of Tables 1 and 2. Blood samples were drawn by cardiac puncture; Quick one-stage activity was assayed using Simplastin Reagent (General Diagnostics).

Plasma warfarin concentrations were assayed by a fluorometric method [19]. Though this assay does not discriminate between warfarin and certain of its metabolites, their concentration in plasma is sufficiently low [20, 21] that no significant interference is expected. High concentrations of ticrynafen were found to quench warfarin fluorescence due to an inner filter effect; however, inclusion of an internal warfarin standard indicated no significant quenching in the test samples. [^{3}H]Vitamin K and vitamin K 2,3-epoxide were extracted from samples of plasma and liver homogenates by chloroform–methanol treatment [22]. The extracts were analyzed by thin-layer chromatography. Recovery of added vitamin K or vitamin K epoxide standards was identical and greater than 90%. Vitamin K and its epoxide accounted for all of the extracted radioactivity.

DT-diaphorase (NAD(P)H: quinone dehydrogenase, EC 1.6.99.2) was purified from vitamin K-deficient rat liver cytosol as described by Wallin [23] and was assayed spectrophotometrically using dichlorophenol-indophenol (DCPIP) as the electron

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acceptor. Reactions were run at 27° in a total volume of 1 ml containing 14 μM DCPIP, 8.5 mg/ml Tween-20, 0.05 M Tris–HCl, pH 7.4, enzyme, and NADH. Reduced vitamin K (KH_2)-dependent and [NADH + vitamin K]-dependent peptide carboxylations were assayed in a total volume of 0.75 ml containing 0.4 ml of vitamin K-deficient solubilized microsomes, 1 mM dithiothreitol (DTT), 0.025 M imidazole–HCl, pH 7.2, 0.25 M sucrose, 0.5 M KCl, 0.5 mM Phe-Leu-Glu-Glu-Leu, 10 μCi sodium [^{14}C]bicarbonate, and 15 μM vitamin KH_2 or vitamin K plus 100 μM NADH and were incubated for 20 min at 17°. Dithiothreitol driven, [DTT + vitamin K]-dependent, carboxylation of endogenous clotting factor precursors was assayed in a total volume of 0.375 ml containing 0.2 ml of vitamin K-deficient whole microsomes, 50 μl of an ATP-generating system plus cycloheximide, 0.25 M sucrose, 0.025 M imidazole–HCl, pH 7.2, 5 μCi sodium [^{14}C]bicarbonate, 1 mM DTT, and 15 μM vitamin K, incubated for 15 min at 27°. Vitamin K epoxide reductase was assayed in a total volume of 0.75 ml containing 0.5 ml whole microsomes, 0.025 M imidazole–HCl, pH 7.2, 0.25 M sucrose, 1 mM DTT, and 20 μM vitamin K epoxide, incubated for 30 min at 27°. Quantitation of vitamin K formed was performed by a high pressure liquid chromatography (HPLC) method previously described [24]. All assays were conducted under conditions yielding valid measurement of the initial velocities; substrates were subsaturating at concentrations close to their apparent K_m values. Microsomal enzyme assays were conducted in a rotary shaker water bath and were open to the air. All results are presented as means ± S.E.M. P values refer to significance in Student's *t*-test.

Vitamin K and vitamin K epoxide were purified by preparative reverse phase HPLC before use. Sodium warfarin was from Endo Laboratories (Garden City, NY), dithiothreitol from Calbiochem (San Diego, CA), ticrynafen (free acid, Selacryn) and its tris salt from Smith Kline & French (Phildelphia, PA), Phe-Leu-Glu-Glu-Leu from Vega Fox (Tucson, AZ), and sodium [^{14}C]bicarbonate (57 mCi/mmol) from Amersham/Searle (Arlington Heights, IL). Vitamin K, NADH, DCPIP, and Tween-20 were from the Sigma Chemical Co. (St. Louis, MO). The [^{3}H]vitamin K used, [5, 6, 7, 8-^{3}H]phylloquinone, was a gift of Dr. Charles M. Siegfried, Department of Biochemistry, University of Nebraska College of Medicine, Omaha, NF.

RESULTS

Coagulation studies. To determine whether the rat is a suitable experimental model for the potentiation of warfarin action by ticrynafen, rats were maintained on a low dose of warfarin until a stable hypoprothrombinemia was obtained. Initiation of ticrynafen treatment while continuing warfarin administration resulted in a further decrease in prothrombin complex activity (Table 1). This effect was observed at both moderate and severe responses to warfarin treatment alone. In trial B, seven of ten rats receiving ticrynafen died after cardiac puncture, whereas only two of the nine warfarin-only rats died. Plasma warfarin concentrations determined in trial B were lower in the ticrynafen-treated rats than in the group receiving warfarin alone. Ticrynafen administration alone had no effect on plasma prothrombin complex activity.

Effects on vitamin K metabolism. Warfarin administration has been shown to alter the ratio of plasma vitamin K and vitamin K 2,3-epoxide in both man

Table 1. Effects of ticrynafen and warfarin on prothrombin complex* and plasma warfarin concentrations†

	Prothrombin complex (% normal)		[Warfarin] (μg/ml)	
Trial‡	Warfarin alone	Warfarin + ticrynafen	Warfarin alone	Warfarin + ticrynafen
A	52.1 ± 6.0	26.7 ± 2.3		
	$P < 0.005$			
B	15.4 ± 2.3	8.8 ± 1.1	0.45 ± 0.03	0.27 ± 0.06
	$P < 0.01$		$P < 0.01$	

* Plasma prothrombin times were determined using Simplastin Reagent and are expressed as percent dilutions of pooled normal rat plasma which would yield equivalent clotting times.

† Total plasma warfarin was assayed by a fluorometric method as described in Materials and Methods.

‡ Trial A: One ppm sodium warfarin was supplied in the drinking water for 7 days. Ticrynafen was given (20 mg per rat each time) by gavage at 8:00 a.m., 12:00 noon and 4:00 p.m. on day 6 and at 8:00 a.m., 10:00 a.m., 12:00 noon, 2:00 p.m., and 4:00 p.m. (10 mg/each) on day 7, as a suspension in sucrose. Blood was drawn at 6:00 p.m. on day 7. Trial B: Warfarin was provided as in Trial A. Ticrynafen (20 mg per rat at each time) was given by gavage at 8:00 a.m., 12:00 noon, and 4:00 p.m. on days 5–7 as a solution in ethanol. Blood was drawn at 8:00 p.m. on day 7. Warfarin consumption measured for this trial was 94 ± 33 μg/kg during the dark cycle and did not differ between the groups before or after institution of ticrynafen treatment. Though the warfarin treatment was nominally the same in these two trials, it is apparent that the effective dose received differed. Results are presented as mean ± S.E.M. (N = eight to ten rats per group in both trials).

Table 2. Effects of warfarin and ticrynafen on vitamin K metabolism

	% Recoveries of administered dose of [^{3}H]vitamin K*		
	Treatment†		
	No drugs	Warfarin	Warfarin + ticrynafen
Plasma			
Vitamin K epoxide	0.10 ± 0.01	0.16 ± 0.03	0.22 ± 0.03
	$P < 0.2$		$P < 0.1$
Vitamin K	0.34 ± 0.01	0.36 ± 0.05	0.36 ± 0.04
Ratio KO/K	0.30 ± 0.05	0.43 ± 0.03	0.61 ± 0.06
	$P < 0.05$		$P < 0.025$
Liver			
Vitamin K epoxide	3.4 ± 1.2	6.3 ± 0.5	7.4 ± 0.7
	$P < 0.05$		$P < 0.2$
Vitamin K	20.7 ± 6.9	20.9 ± 3.4	19.1 ± 1.1
Ratio KO/K	0.16 ± 0.10	0.31 ± 0.02	0.39 ± 0.03
	$P < 0.01$		$P < 0.1$

* Samples were extracted by chloroform–methanol treatment, and the amounts of [^{3}H]vitamin K and [^{3}H]vitamin K epoxide were determined as described in Materials and Methods. Results are presented as mean ± S.E.M. (N = three to four rats per group).

† Rats were given 0.12 mg/kg sodium warfarin, i.p., at zero time. After 30 min, 7.5 μg (5 μCi) of [^{3}H]vitamin K_1 in 50% ethanol was administered, i.c. At 30, 90, and 150 min, ticrynafen (20 mg per rat each time) was given. Plasma samples were drawn by cardiac puncture, and livers were excised after 210 min.

[25, 26] and animals [27]. When either warfarin alone or warfarin plus ticrynafen was given to rats, the recovery of [^{3}H]vitamin K remaining in plasma 210 min after administration did not differ from that of control animals (Table 2). Neither drug appears to interfere with clearance of vitamin K from plasma. Recoveries from liver, the principal site of vitamin K uptake [27], also did not differ.

Plasma and liver recoveries of [^{3}H]vitamin K epoxide were increased in rats receiving warfarin compared to controls, and they were increased further in rats receiving both warfarin and ticrynafen. Expression of the data as vitamin K epoxide to vitamin K ratios minimized animal-to-animal variation caused by alterations in extraction efficiency and sampling, and treatment differences expressed in this form were statistically more significant. This effect of ticrynafen on vitamin K epoxide to vitamin K ratios was seen when 0.12 mg/kg warfarin was given. At a higher warfarin dose (0.25 mg/kg) which resulted in higher epoxide to vitamin ratios, no effect of ticrynafen was observed (KO/K = 1.05 ± 0.16 and 1.14 ± 0.12 in plasma, and 0.41 ± 0.03 and 0.46 ± 0.04 in liver, for warfarin alone and warfarin plus ticrynafen treatments respectively). Plasma warfarin concentrations determined for the higher warfarin dosage were unaltered by ticrynafen treatment (warfarin alone: 1.04 ± 0.05 μg/ml plasma; warfarin + ticrynafen: 0.95 ± 0.08 μg/ml).

Assays of vitamin K-dependent enzymes in vitro. The activity of several vitamin K-dependent enzymes was assayed over a range of ticrynafen concentrations. The concentration of ticrynafen giving 50% inhibition of activity was determined from plots of percent activity vs log ticrynafen for six data points bracketing the 50% value by two orders of magnitude. A similar approach was used to determine the concentrations of warfarin yielding 50% inhibition. The influence of warfarin on enzyme activity in the presence of a concentration of ticrynafen which gave approximately 50% inhibition of the control activity was then examined. The results are summarized in Table 3. In all cases, a plot of the percent inhibition of enzyme activity by warfarin in the presence of ticrynafen was parallel to that for warfarin alone.

Except in the case of DT-diaphorase, Dixon plots ($1/v$ vs [inhibitor]) were non-linear, indicating that neither drug inhibits the enzymes by acting at a single site and/or that there are multiple binding sites for the drugs. This is not surprising in view of the crude enzyme preparations used in these assays which preclude a more sophisticated treatment of the data. However, it is clear that ticrynafen does not appreciably potentiate the action of warfarin on any of the enzymes. The concentration of warfarin needed to inhibit the residual activity in the presence of ticrynafen is essentially identical to that needed to inhibit the control activity. The two drugs appear to act independently.

Inhibition of purified DT-diaphorase. Of the enzymes tested, only DT-diaphorase was sensitive to low levels of ticrynafen. The inhibition of this enzyme by ticrynafen and warfarin was examined in some detail. Ticrynafen is metabolized by a reduction of the carbonyl to an alcohol [28–30] with a midpoint potential of −1.17 V vs Standard Calomel Electrode (SCE) at pH 8.0 [31]. Based on absorbance changes at either the ticrynafen absorbance maximum 287 nm or at that of NADH at 340 nm, ticrynafen was not a substrate for DT-diaphorase. It did not react with NADH non-enzymatically, nor did it interfere with the assay by facilitating reoxidation of DCPIP.

The steady-state kinetics of DT-diaphorase follow a classic ping-pong mechanism with substrate inhibition by high concentrations of DCPIP, and its inhibition by coumarins is competitive vs NADH [32–34]. The activity of DT-diaphorase was measured at various NADH concentrations for several fixed levels of ticrynafen. Lineweaver–Burk plots of these data (Fig. 1) intersected on the $1/v$ axis, show-

Table 3. Concentrations of inhibitor yielding 50% inhibition of activity *in vitro*

Activity*	IC_{50} [Ticrynafen]	[Win]	In the presence of ticrynafen† [Warfarin]
Cytosolic DT-diaphorase	8 μM	34 μM	34 μM
Vitamin KH_2-dependent peptide carboxylation	3 mM	1.5 mM	1.0 mM
[Vitamin K + NADH]-dependent peptide carboxylation	2.4 mM	2.3 mM	1.5 mM
[Vitamin K + DTT]-dependent protein carboxylation	10 mM	1.5 μM	1.0 μM
Vitamin K epoxide reductase	10 mM	2 μM	2 μM

* Cytosolic DT-diaphorase activity of the purified enzyme was assayed at 100 μM NADH and 14 μM DCPIP. Carboxylase and vitamin K epoxide reductase assays were conducted as described in Materials and Methods.

† [Warfarin] giving 50% inhibition of the residual activity in the presence of a [ticrynafen] giving approximately 50% inhibition of the control activity: for diaphorase, ticrynafen of 8 μM gave 55% inhibition; for KH_2-driven carboxylation, 3 mM gave 32% inhibition; for NADH-driven carboxylation, 3 mM gave 64% inhibition; for epoxide reductase, 5 mM gave 25% inhibition; and for DTT-driven carboxylation, 5 mM gave 30% inhibition.

ing that ticrynafen is competitive with NADH. A replot of the slopes vs ticrynafen (inset Fig. 1) gave a straight line, showing that only one ticrynafen molecule binds per NADH binding site. The apparent K_i for ticrynafen at fixed (14 μM) DCPIP was 1.3 μM. For comparison, the K_i for warfarin under the same conditions was 8 μM, and the K_m for NADH was 38 μM. The inhibition of DT-diaphorase at fixed NADH and DCPIP concentrations was examined for various concentrations of warfarin at several fixed ticrynafen concentrations. Plots of either $1/v$ vs warfarin (Fig. 2A) or $1/v$ vs ticrynafen (Fig. 2B) gave a series of parallel straight lines. This pattern is indicative of mutually exclusive binding of both inhibitors to a single binding site [35]. No enzyme–ticrynafen–warfarin complexes were observed.

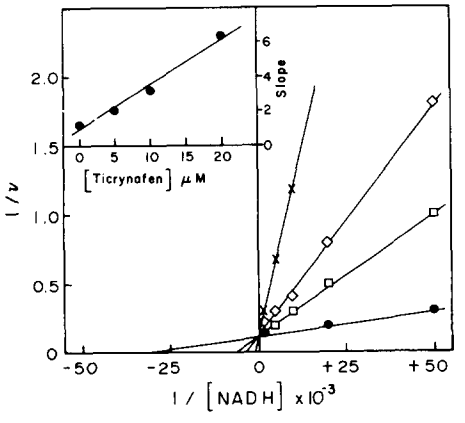

Fig. 1. Competitive inhibition of cytosolic DT-diaphorase by ticrynafen. Double-reciprocal plot of activity ($1/\Delta A_{600}$/min × 10^{-2}) vs concentration of NADH ($1/[NADH]$ $M^{-1} \times 10^{-3}$) at (●) 0, (□) 5, (◇) 10, and (×) 20 μM ticrynafen. Assayed using 15 μM DCPIP as the electron acceptor. Inset: Replot of slopes ($M/\Delta A_{600}$/min × 10^3) vs ticrynafen concentration.

DISCUSSION

These studies demonstrate that ticrynafen potentiation of warfarin anticoagulant action can be observed in the rat and assess the basis for this response. Both enhanced warfarin-dependent depression of coagulation factor activity, and increased frequency of hemorrhagic incidents occurred. There is clearly a pharmacokinetic component to the potentiation observed in humans [8]; however, our results are contrary to increased warfarin concentrations as the primary mode of potentiation in the rat. Plasma warfarin concentrations were decreased after several days of ticrynafen administration, and were unchanged for brief treatment with both drugs, indicating that additional mechanisms of potentiation may occur. There are differences in the pharmacokinetics and metabolism of both drugs in humans [1, 30–31, 36] and in rats [37–40] which could influence the observed responses.

Warfarin administration increases the ratio of vitamin K epoxide to vitamin K in both plasma and liver by inhibiting the liver vitamin K-epoxide reductase [27]. At the lower warfarin dose used in this study, ticrynafen enhanced the warfarin-dependent increase in the vitamin K epoxide to vitamin K ratios. At higher warfarin doses, this was not observed. These results suggest that ticrynafen enhances inhibition of epoxide reduction for warfarin doses yielding only partial inhibition of this enzyme. In addition to the vitamin K-epoxide reductase, liver microsomes contain a DTT-dependent quinone reductase, and recent evidence [41] clearly demonstrates that both of these activities are pharmacologically-important sites of anticoagulant action. No significant effect of ticrynafen on the activity of vitamin K epoxide reductase or [vitamin K + DTT]-dependent carboxylation or on their inhibition by warfarin was observed in this study. Therefore, potentiation of

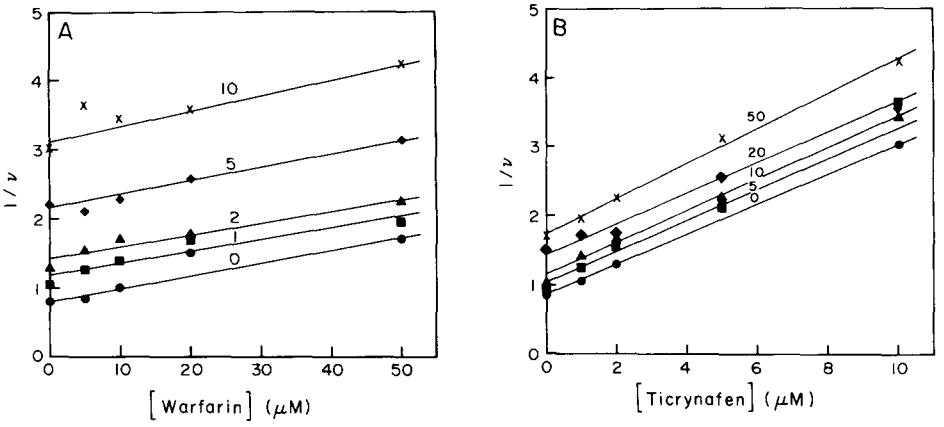

Fig. 2. Double inhibition study. Reciprocal of DT-diaphorase activity ($1/\Delta A_{600}$/min × 10^{-2}) vs concentration of warfarin at (●) 0, (■) 1, (▲) 2, (◆) 5, and (×) 10 μM ticrynafen (Fig. 2A). Reciprocal of activity vs ticrynafen concentration at (●) 0, (■) 5, (▲) 10, (◆) 20, and (×) 50 μM warfarin (Fig. 2B). Assayed using 100 μM NADH and 14 μM DCPIP.

coumarin anticoagulant action and inhibition of epoxide reduction *in vivo* must be the result of ticrynafen action at a different site(s), possibly the endogenous supply of reductant replaced by DTT *in vitro*.

DT-diaphorase was found to be very sensitive to ticrynafen inhibition *in vitro*. The physiological role of this enzyme is still unclear. Multiple differentially inducible forms of this enzyme occur [42–45], and the activity is widely distributed among the subcellular compartments [46, 47]. The activity is induced by a variety of carcinogens [46] and may function in detoxification [47]. Mitochondria contain significant DT-diaphorase which may function in alternate respiratory pathways. Inhibition of these functions of diaphorase may be responsible for the hepatotoxicity of ticrynafen which has been reported. A role for DT-diaphorase in vitamin K function was proposed initially on the basis of its sensitivity to coumarin anticoagulants. Vitamin K-dependent carboxylation requires vitamin KH_2 as a substrate, and *in vitro* vitamin KH_2 may be added exogenously or generated *in situ* from vitamin K quinone and either NADH or DTT [48]. Microsomes contain at least two NADH dehydrogenases capable of reducing vitamin K. One appears to be identical with cytosolic DT-diaphorase which will reduce membrane-bound vitamin K [49] and is capable of reconstituting [NADH + vitamin K]-dependent carboxylation in dehydrogenase-depleted microsomes [50]. However, differences in the influence of riboflavin status on cytoplasmic DT-diaphorase compared to [vitamin K + NADH]-dependent carboxylation [18] and only partial inhibition of the latter by antibody against purified cytosolic diaphorase [51] indicate that another dehydrogenase(s) is also significant in microsomal vitamin K reduction.

The relative importance of the various vitamin K reductase pathways, in the reduction of vitamin K *in vivo*, has been difficult to assess. The induction of DT-diaphorase by vitamin K deficiency suggests a role for this enzyme as vitamin K reduction becomes limiting [18]. Failure to observe ticrynafen inhibition of [vitamin K + NADH]-dependent carboxylation *in vitro* does not rule out depressed carboxylation *in vivo* by inhibition of DT-diaphorase reduction of vitamin K, as the reduction step may well not be rate limiting under these assay conditions. Inhibition of this function of diaphorase under conditions of effective vitamin K deficiency (produced by anticoagulant inhibition of vitamin K epoxide recycling) would yield depressed synthesis of coagulation factors through depletion of vitamin K hydroquinone.

Multiple mechanisms of ticrynafen–coumarin potentiation may occur, and their relative importance need not be the same in various species. The data presented here indicate that ticrynafen and warfarin do not interact synergistically at the molecular level but, rather, that a separate additive inhibition of vitamin K-dependent activities causes a potentiation of the response which is observed for warfarin alone. Possible mechanisms apparent from this study are interference with vitamin K reduction and interference with recycling of vitamin K epoxide. Both appear to result from inhibition of DT-diaphorase which is very sensitive to both ticrynafen and coumarin anticoagulant inhibition *in vitro*. Additional influences on coumarin metabolism and pharmacokinetics may occur. These effects may occur to varying extents depending on species, vitamin K status, and additional factors. Nutritional status, additional drug treatments, genetic predisposition, and liver disease were uncontrolled variables in most of the reported cases of ticrynafen–coumarin interaction and hepatotoxicity in humans and are known to influence the action of coumarin anticoagulants alone.

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REFERENCES

1. M. Nemati, M. C. Kyle and E. D. Freis, *J. Am. med. Ass.* **237**, 652 (1977).

2. R. M. Stote, J. W. Dubb, R. G. Familiar and F. Alexander, *Clin. Pharmac. Ther.* **23**, 456 (1978).
3. J. G. R. de Carvalho, F. G. Dunn, S. G. Chrysant and E. D. Frohlich, *Ann. intern. Med.* **138**, 53 (1978).
4. M. Detilleux, R. Caquet and C. Laroche, *Nouv. Presse med.* **5**, 2395 (1976).
5. A. B. Grand, B. Drouin and G. J. Arche, *Nouv. Presse Med.* **6**, 2691 (1977).
6. H. Portier, F. Destaing and L. Chauve, *Nouv. Presse Med.* **6**, 468 (1977).
7. D. A. McLain, F. J. Garriga and O. S. Kantor, *J. Am. med. Ass.* **243**, 763 (1980).
8. R. A. O'Reilly, *Clin. Pharmac. Ther.* **32**, 356 (1982).
9. R. A. O'Reilly, *A. Rev. Med.* **27**, 245 (1976).
10. J. T. Slattery and G. Levy, *J. pharm. Sci.* **68**, 393 (1979).
11. J. Prandota, E. Albengres and J. P. Tillement, *Int. J. clin. Pharmac. Ther. Toxic.* **18**, 158 (1980).
12. P. Groussin, D. Chavanne, J. P. Renard, M. Medelsi, B. Arbiele-Brassard and D. Royere, *Méd. Chir. Dig.* **8**, 463 (1979).
13. D. Mechali, C. Marche, J. Metayer, G. Saimot, S. Matheron, W. Rozenbaum and J-P. Coulaud, *Thérapie* **35**, 197 (1980).
14. O. Bousquet, Th. Saigot, P. Bernard, J. L. Gerbal and A. Sarrazin, *Thérapie* **35**, 205 (1980).
15. C. Eugene, C. Patriarche, A. Bergue and J. Quevauvilliers, *Nouv. Presse Med.* **9**, 1885 (1980).
16. W. R. Barclay, *J. Am. med. Ass.* **243**, 771 (1980).
17. R. Poupon, J. C. Homberg, N. Abuaf, J. Petit, F. Bodin and F. Darnis, *Nouv. Presse Med.* **9**, 1881 (1980).
18. P. C. Preusch and J. W. Suttie, *J. Nutr.* **11**, 2087 (1981).
19. M. Corn and R. Berberich, *Clin. Chem.* **13**, 126 (1967).
20. R. Laliberte, S. Chakrabarti and J. Brodeur, *J. Pharmac. exp. Ther.* **200**, 44 (1977).
21. M. J. Fasco, M. J. Cashin and L. S. Kaminsky, *J. Liquid Chromat.* **2**, 565 (1979).
22. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
23. R. Wallin, *Biochem. J.* **181**, 127 (1979).
24. M. J. Fasco, P. C. Preusch, E. Hildebrandt and J. W. Suttie, *J. biol. Chem.* **258**, 4372 (1983).
25. M. J. Shearer, A. McBurney and P. Barkhan, *Br. J. Haemat.* **24**, 471 (1973).
26. M. J. Shearer, A. McBurney, A. M. Breckenridge and P. Barkhan, *Clin. Sci. molec. Med.* **52**, 621 (1977).
27. R. G. Bell, *Fedn Proc.* **37**, 2599 (1978).
28. J. C. Levron, J. M. Le Fur, P. Adnot, Y. Dormard, N. Cucumel and G. Enjoubault, *Eur. J. Drug Metab. Pharmacokinet.* **2**, 107 (1977).
29. J. W. Dubb, A. R. Maass, R. G. Familiar, R. M. Stote, B. V. Hwang and F. Alexander, *Post-grad. med. J.* **55**, 47 (1979).
30. T. G. Murray, S. Brown, J. W. Dubb, B. Y. Hwang and R. M. Stote, *Clin. Pharmac. Ther.* **27**, 686 (1980).
31. F. Pellerin, J-F. Letavernier, N. Chanon and T. Lezian, *Eur. J. Med. Chem. Chim. Ther.* **12**, 477 (1977).
32. S. Housuda, W. Nakamura and K. Hayashi, *J. biol. Chem.* **249**, 6416 (1974).
33. P. M. Hollander and L. Ernster, *Archs Biochem. Biophys.* **169**, 560 (1975).
34. P. M. Hollander, T. Bartfai and S. Gatt, *Archs Biochem. Biophys.* **169**, 568 (1975).
35. W. W. Cleland, *Adv. Enzymol.* **45**, 273 (1977).
36. R. M. Stote, A. R. Maass, D. A. Cherrill, M. M. A. Beg and F. Alexander, *J. Pharmac. Clin.* (special issue), 19 (1976).
37. A. Zimmerman and J. T. Matschiner, *Biochem. Pharmac.* **23**, 1033 (1974).
38. Y. Dormard, J-C. Levron, P. Adnot, T. Lebedeff and G. Enjoubault, *Eur. J. Drug Metab. Pharmacokinet.* **1**, 41 (1976).
39. S. Minor, K. Taylor, T. F. Knight, H. O. Senekjian and E. J. Weinman, *Res. Commun. Chem. Path. Pharmac.* **25**, 53 (1979).
40. C. Oker-Blom, J. Makinen and G. Gothoni, *Toxic. Lett.* **6**, 93 (1980).
41. M. J. Fasco, E. F. Hildebrandt and J. W. Suttie, *J. biol. Chem.* **257**, 11210 (1982).
42. M. Raftell and K. Blomberg, *Archs Biochem. Biophys.* **199**, 165 (1980).
43. Y. H. Edwards, J. Potter and D. A. Hopkinson, *Biochem. J.* **187**, 429 (1980).
44. B. Hojeberg, K. Blomberg, S. Stenberg and C. Lind, *Archs Biochem. Biophys.* **207**, 205 (1981).
45. C. Lind and B. Hojeberg, *Archs Biochem. Biophys.* **207**, 217 (1981).
46. A. M. Benson, M. J. Hunkeler and P. Talalay, *Proc. natn. Acad. Sci. U.S.A.* **77**, 5216 (1980).
47. C. Lind and L. Ernster, *Biochem. biophys. Res. Commun.* **56**, 392 (1974).
48. J. W. Suttie, *CRC Crit. Rev. Biochem.* **8**, 191 (1980).
49. C. Martius, R. Ganser and A. Viviani, *Fedn Eur. Biochem. Soc. Lett.* **59**, 13 (1975).
50. E. Wallin, O. Gebhardt and H. Prydz, *Biochem. J.* **169**, 95 (1978).
51. R. Wallin and S. Hutson, *J. biol. Chem.* **257**, 1583 (1982).