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Phenylbutazone-warfarin interaction in the dog

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The administration of phenylbutazone together with warfarin to dogs resulted in an elevation of the free fraction of warfarin in the plasma from 2·6 to 8·0% thus providing direct support for the notion that phenylbutazone induced inhibition of warfarin binding to plasma proteins. This inhibition as evaluated by a kinetic method was accompanied by a two-fold decrease in the plasma half-life of warfarin from 18·4 h in control animals to 9·6 h in phenylbutazone-treated animals. Marked increases in warfarin-induced hypoprothrombinaemia were observed when at doses up to 8 mg kg^{-1} (orally) it was given with phenylbutazone (50 mg kg^{-1}, orally). The unbound fraction of warfarin in canine plasma ranged from 1·7 to 4·3% indicating individual differences in the extent of the plasma binding of warfarin in the dog.

The predominant mechanism by which pyrazolone compounds appear to enhance coumarin-induced hypoprothrombinaemia is one of displacement of coumarins from albumin binding sites in plasma by competitive binding (O'Reilly & Aggeler, 1968; O'Reilly & Levy, 1970; Solomon & Schrogie, 1967; Jun, Luzzi & Hsu, 1972), although some evidence suggests displacement from protein-bound depots in other tissues as well (Wosilait & Eisenbrandt, 1972). However, despite the relative abundance of *in vitro* and presumptive *in vivo* evidence to support this notion, there is no direct *in vivo* evidence for an increase in the unbound fraction of coumarin anticoagulant after the concurrent administration of phenylbutazone or oxyphenbutazone. We now provide evidence that phenylbutazone is capable of elevating the free fraction of warfarin when the two drugs are administered together to dogs.

MATERIALS AND METHODS

Male, mongrel dogs (Blue Farms, Plain City, Ohio) weighing 10-20 kg were sampled for blood by the technique of Hovell (1968) from the jugular vein. Whole blood was drawn into either oxalated or EDTA-anticoagulated evacuated tubes (Vaccutainer, Beckton-Dickinson, Rutherford, New Jersey) to a final sample volume of 3 or 10 ml, respectively. Animals were maintained on a diet of Purina Dog Chow (laboratory grade) and fed at 1.00 p.m. daily. Water was freely available. Warfarin was administered at 9.00 a.m.; phenylbutazone at 9.00 a.m. and 9.00 p.m.

Warfarin determinations. Total plasma warfarin was measured by a spectrofluorometric method modified from the method of Lewis, Ilnicki & Carlstrom (1970) in which the warfarin spot after thin-layer chromatography is eluted with 4·0 ml acetone. Three ml of the eluate was taken to dryness under nitrogen and the residue dissolved and fluorescence read in 2·0 ml of *NN*-dimethylformamide at excitation and emission

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wavelengths of 330 and 408 nm, respectively. The free fraction of warfarin was estimated in a similar fashion after ultrafiltration. Ultrafiltration was accomplished by positive pressure filtration (nitrogen) of 10 ml plasma samples through a Diaflo PM–10 membrane, retentive for molecules exceeding 10 000 mol wt (Amicon). One ml of ultrafiltrate was collected and assayed for the free fraction of warfarin. Values for unbound warfarin were corrected for non-specific membrane-binding which was estimated by passing protein-free solutions of warfarin (pH 7·4) through the membrane and measuring the warfarin concentration in the ultrafiltrate. Ultrafiltrate concentrations of warfarin were one-half those of the parent protein free solution regardless of the initial warfarin concentration used. The extent of non-specific membrane binding of warfarin remained unchanged even when phenylbutazone in concentrations exceeding warfarin by fifty times was admixed with warfarin.

Prothrombin determinations. Plasma prothrombin complex activity was determined by the modified method of Quick (1966) and expressed as a percent of normal. Each animal's experimental prothrombin activities were calculated on the basis of its own normal value. Hypoprothrombinaemic responses were expressed as rates of prothrombin complex synthesis (R_{syn}) and derived by the kinetic method of Nagashima, O'Reilly & Levy (1968).

Dosing regimens. To study the influence of phenylbutazone upon warfarin-induced hypoprothrombinaemia and the plasma half-life of warfarin, (±)-warfarin sodium as the commercially available tablet (Endo) was administered orally to dogs at 0·8 mg kg^{-1} or together with 50 mg kg^{-1} of phenylbutazone (Geigy). Plasma for both prothrombin activity and warfarin determinations was collected at the time of dosing and at 3, 6 and 12 h subsequently. Thereafter, plasma was collected at 6, 12 or 24 h intervals until prothrombin values approached pre-medication values.

For the evaluation of the extent of the inhibition of *in vivo* warfarin binding, animals were initially dosed with sodium warfarin (1·0 mg kg^{-1}) intravenously. Plasma concentrations of the total and unbound fractions of warfarin were estimated in blood samples withdrawn 60 min after warfarin administration. After one week, the animals were administered 25 mg kg^{-1} phenylbutazone (Jensen-Salsbery Laboratories, Kansas City, Missouri) by jugular venipuncture. Sixty min subsequent to infusion of phenylbutazone, sodium warfarin was infused at 1·0 mg kg^{-1} and samples were taken 60 min post-infusion.

RESULTS

Phenylbutazone-induced changes in hypoprothrombinaemic responses to warfarin and warfarin half-life.

The disappearance of prothrombin complex activity from the plasma of animals treated with a single prothrombin synthesis-blocking doses of warfarin (0·8 mg kg^{-1}, orally) was apparently a first-order process. The rate constant for the dissappearance of prothrombin (complex) activity is derived from the slope of the disappearance curve, plotted as ln pro-time *vs* time (days) is:

$$y = -1{\cdot}41(x) + 4{\cdot}54$$

where y = ln pro-time, x = days, −1·41 = slope = k_d (rate constant for disappearance of prothrombin complex activity), 4·54 = y — intercept. The disappearance of prothrombin activity from plasma after warfarin was unchanged when a single oral dose of phenylbutazone (50 mg kg^{-1}) was administered with the warfarin.

The relationship *in vivo* between the rate of synthesis of prothrombin activity and the plasma concentration of warfarin is depicted for each of four dogs in Fig. 1. Synthesis rates decrease with increasing plasma concentrations of the drug. When warfarin is administered in a single oral dose (0·8 mg kg^{-1}), the apparent plasma concentrations which are required to effect inhibition of prothrombin synthesis are higher than when warfarin is administered with phenylbutazone (50 mg kg^{-1}). The

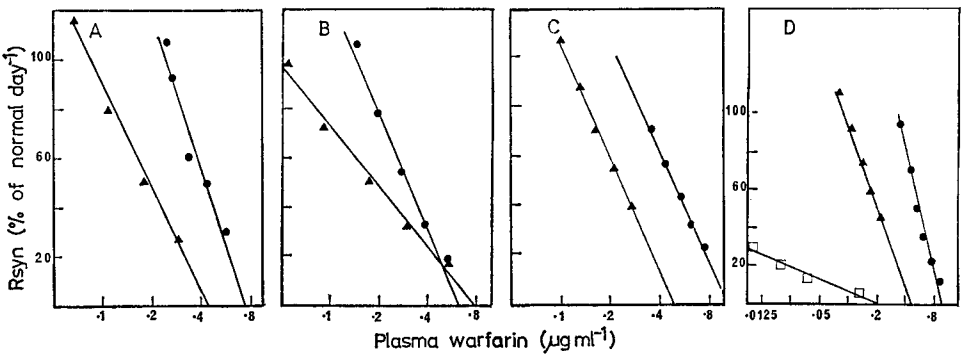

FIG 1, A–D. Influence of plasma warfarin concentration upon rate of prothrombin complex activity synthesis. Warfarin (0·8 mg kg^{-1}) was administered orally with and without phenylbutazone (50 mg kg^{-1}). In one instance (D) phenylbutazone administration was continued every 6 h with warfarin until prothrombin complex activity returned to normal. Each graph represents a different dog. ● Warfarin. ▲ Warfarin + phenylbutazone. □ Warfarin + phenylbutazone (chronic).

potentiation is evidenced by a shift-to-the-left of the plasma concentration-response curve. Furthermore, the continued administration of phenylbutazone every 6 h until prothrombin complex activity returned to normal values, even further reduced those concentrations of warfarin required to inhibit prothrombin synthesis *in vivo* resulting in a dramatic shifting of the plasma concentration-response curve (Fig. 1D).

An apparent two-fold decrease in the warfarin plasma half-life is also associated with the concurrent administration of single doses of warfarin and phenylbutazone (Table 1), and is consistent with the notion of displacement of protein-bound warfarin.

Protein-binding inhibition in dog plasma in vivo

The intravenous administration of phenylbutazone (25 mg kg^{-1}) 60 min before the intravenous administration of sodium warfarin (1·0 mg kg^{-1}) increased the plasma concentration of the unbound warfarin fraction. The unbound warfarin fraction

Table 1. *Effect of acute phenylbutazone administration on warfarin plasma half-life (h).*

Animal	Control[1]	Phenylbutazone[2]
1	21·0	11·4
2	13·0	7·2
3	23·0	11·2
4	16·5	8·7
Mean (n = 4)	18·4	9·6*
s.e.m.	2·3	1·0

[1] Single oral dose of warfarin (0·8 mg kg^{-1}).
[2] Single oral dose of warfarin (0·8 mg kg^{-1}) and phenylbutazone (50 mg kg^{-1}).
* Significantly different from control ($P < 0·01$) by Student's *t*-test.

comprised 2·6% of the total warfarin concentration when no other drug was present in the plasma. The prior administration, however, of phenylbutazone resulted in an approximate three-fold increase in the concentration of unbound warfarin (Table 2).

Table 2. *Effect of phenylbutazone (PBZ) on the availability of warfarin in the dog.* Controls were injected with sodium warfarin in saline (10 mg ml^{-1}) with a dose of 1 mg kg^{-1} into the jugular vein. Plasma concentrations of total and free warfarin were determined from samples drawn 60 min after dosing. Phenylbutazone-treated animals had in addition an infusion of 25 mg kg^{-1} drug which preceded warfarin by 60 min.

	Control			PBZ			%Free PBZ
Animal	C_t[1]	C_f[2]	%Free	C_t	C_f	%Free	%Free control
4	6·76	0·12	1·7	6·38	0·40	6·2	3·6
1	7·04	0·16	2·2	5·79	0·48	8·2	3·7
3	7·65	0·18	2·3	7·51	0·58	7·7	3·3
2	5·54	0·24	4·3	5·24	0·52	9·9	2·3
Mean (n = 4)	6·75	0·18	2·6	6·23	0·50	8·0 *	3·2
s.e.m.	0·44	0·03	0·6	0·49	0·04	0·8	0·3

[1] Total plasma warfarin (μg ml^{-1}).
[2] Free (unbound) plasma warfarin (μg ml^{-1}). Values have been corrected for warfarin adsorbance to ultrafiltration membrane.
* Significantly different from control ($P < 0·005$) by Student's *t*-test.

DISCUSSION

The present results further substantiate the notion of competitive displacement of warfarin from plasma proteins as one of the mechanisms by which phenylbutazone enhances the anticoagulant response to warfarin.

Phenylbutazone has no direct effects upon the normal formation and degradation of vitamin K-dependent clotting factors (Brodie, Lowman & others, 1954). Single doses administered with single doses of warfarin reduced the total plasma concentrations of warfarin necessary to effect a given hypoprothrombinaemic response in the dog. This was observed as a distinct shift-to-the-left of the plasma concentration-response curve which is presumptive evidence for changes in warfarin distribution related to the simultaneous administration of phenylbutazone (O'Reilly & Levy, 1970; Jahnchen, Wingard & Levy, 1973). Such a shift was also reported in man after a chronic pretreatment with phenylbutazone (O'Reilly & Levy, 1970). After a single dose of phenylbutazone and warfarin given together to the dog, phenylbutazone disappears at a rapid rate (6 h half-life) from plasma and its effects on inhibition of binding at lower plasma concentrations of warfarin will not be nearly so marked as when it is present in plasma in high concentrations after multiple dosing, when a much more dramatic decrease in slope can be effected (Fig. 1D).

The potentiated response to warfarin associated with phenylbutazone administration was accompanied by a two-fold decrease in the plasma half-life of warfarin, displacement from binding sites making the drug more rapidly available for metabolism and excretion. Wosilait & Eisenbrandt (1972) have already demonstrated that oxyphenbutazone (75 mg kg^{-1}) administered 1 h after warfarin in the rat markedly increases the biliary excretion of warfarin.

Total plasma concentrations of warfarin declined very little (from 6·75 to 6·23 μg ml^{-1}) after phenylbutazone, even though free concentrations roughly trebled.

Thus, it may not necessarily follow that inability of a drug to significantly lower total plasma concentrations of another drug precludes a mechanism of displacement from binding. For example, such an explanation might fit the findings of O'Reilly, Sahud & Robinson (1972) with clofibrate-induced potentiation of the hypoprothrombinaemic response to warfarin.

A nearly four-fold difference exists in the free fraction of warfarin in human and dog plasmas when determined by ultrafiltration (Bachmann, 1974). This difference is in good agreement with the relative differences in warfarin binding constants for human and dog albumin (O'Reilly, 1970), and may contribute to the shorter plasma half-life and apparently greater sensitivity of the dog to the hypoprothrombinaemic effect of warfarin compared to man. The normal value for the free warfarin fraction over the therapeutic range of warfarin plasma concentration in man is 0·6.% This value can be increased to 1·0% with relatively small concentrations of phenylbutazone (40 μg ml^{-1}) (Bachmann, 1974).

Considerable variability in the free fraction of warfarin was observed for this small sampling of dog plasmas. Similar variability has been demonstrated in plasma from rat (Levy & Yacobi, 1974) and man (Bachmann, 1974). The extent to which individual differences in the plasma binding of warfarin contribute to the 33% range in warfarin plasma half-lives in man (Vessell & Shively, 1974) remains to be evaluated.

Finally, Lewis, Trager & others (1974) have recently provided evidence that in man, phenylbutazone potentiates racemic warfarin's hypoprothrombinaemic effect by inhibiting the rate of hepatic metabolism of the more active enantiomer *S*-warfarin. Whether the same phenomenon contributes to the enhancement of warfarin-induced hypoprothrombinaemia in the dog has yet to be established.

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