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PHARMACOKINETICS AND PHARMACODYNAMICS OF WARFARIN AT STEADY STATE

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1 The relationship between warfarin dose, total and free plasma warfarin concentration, and anticoagulant effect was examined at several steady-state levels in fifteen patients during withdrawal of warfarin therapy.

2 Total plasma clearance was significantly correlated with the free fraction in plasma ($r=0.955$).

3 There was an age related decline in the dose of warfarin, and in the total and free plasma warfarin concentrations required to produce the same anticoagulant effect. However, neither total nor free plasma warfarin clearances varied with age.

4 Individual patients' log concentration-effect relationships were linear above a prothrombin ratio of 1.2 and there was a significant correlation ($r=-0.586$) between the slope and the free fraction of warfarin in plasma. It is suggested that plasma protein binding may reflect the interaction between warfarin and its effector site in the hepatocyte.

Introduction

Patients vary widely in the daily doses of warfarin which they require to produce similar anticoagulant effects (Aggeler & O'Reilly, 1966; Smith & Rawlins, 1973). Studies designed to investigate the causes for this variation are rendered complicated by the extensive protein binding of the drug (O'Reilly, 1967; Levy & Yacobi, 1974) and by its delayed anticoagulant action (Nagashima, O'Reilly & Levy, 1969). We have therefore examined the pharmacokinetics of total and free warfarin in plasma under steady-state conditions, and at several different dose levels, in relation to its anticoagulant effects.

Methods

Fifteen ambulant out-patients (seven males) were included in the study. Thirteen patients had been anticoagulated with warfarin as treatment for thrombeombolic disease, and two patients had severe unstable angina pectoris. All patients had been receiving warfarin (Marvan) for at least 4 weeks prior to inclusion in the study, and were taking a dose sufficient to maintain the prothrombin ratio at 1.8 to 2.5 during the previous 3 weeks. None was taking any drug known to interact with warfarin or had evidence of cardiac failure or hepatic disease.

When a clinical decision to discontinue anticoagulant therapy had been made, the patient was asked to take his daily warfarin at 12.00 h throughout the course of the study. At three-weekly intervals venous blood was taken at 10.00 h into sodium citrate for estimation of prothrombin ratio and plasma warfarin concentration. The prescribed daily warfarin dose was then reduced by 0.5 mg/day or 1 mg/day, and maintained at this dose for the next 3 week period. The procedure was repeated every 3 weeks until the prothrombin ratio had fallen to 1.0 or warfarin had been withdrawn.

The prothrombin time was measured by the method of Quick (1938) using thrombokinase (Geigy) and the prothrombin ratio calculated by comparison with the one stage prothrombin time for control plasma (usually 15 s in our laboratory).

Plasma warfarin concentrations were measured in triplicate by a modification of the fluorimetric method of Lewis, Ilnicki & Carlstrom (1970). After extraction into ethyl acetate, warfarin was separated from its metabolites by thin layer chromatography and the fluorescence measured directly by scanning spectrofluorimetry (Perkin-Elmer). Quantitation was achieved by comparing the area of the warfarin peak with that of an internal standard (phenprocoumon) added to the plasma initially. The method was sensitive to 0.05 μg warfarin/ml of plasma.

In fourteen patients, the plasma protein binding of warfarin was measured by equilibrium dialysis of plasma against phosphate buffer (pH 7.38). [^{14}C]-warfarin (Specific Activity 5-15 m Ci/m mol, 99% radiochemically pure at purchase, Amersham, England) was added to the freshly prepared phosphate buffer to achieve a concentration of

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approximately 0.7 μg/ml and 0.5 ml of this buffer was dialysed against 0.5 ml plasma separated by a Visking membrane in a perspex equilibrium dialysis block. Three estimations were performed in each patient and the blocks were horizontally agitated 2 times/s in a water bath at 37°C for 18 h. Preliminary experiments showed that equilibrium between buffer and plasma had been achieved by this time. Radioactivity was measured in 200 μl aliquots of buffer and plasma by liquid scintillation spectrometry.

Total plasma warfarin clearance was estimated using the equation of Wagner, Northam, Alway & Carpenter (1965):

$$\text{Total plasma clearance} = \frac{\text{Dose} \times \text{Fraction of dose absorbed}}{\text{Steady-state total plasma concentration} \times \text{dose interval}} \quad (1)$$

The dosage interval was known to be 24 h. Complete bioavailability of warfarin was assumed (Breckenridge & Orme, 1973) and the total plasma concentration of warfarin at 22 h was assumed to approximate to the mean steady-state concentration of warfarin. Full compliance with therapy was also assumed. Since for each patient total plasma warfarin concentration was measured at several dose levels the average of the individual estimates of clearance was used in the analysis of the results. Free plasma clearance was calculated:

$$\text{Free plasma clearance} = \frac{\text{total plasma clearance}}{\text{free fraction}} \quad (2)$$

Results

The age of the fifteen patients in the study ranged from 33 to 78 years (mean 58.5 years ± 12.6 s.d.). Their warfarin requirements at the beginning of the study varied from 2 mg to 7.5 mg daily (mean 4.6 s.d. ± 1.9 mg). There was a significant negative correlation between the age of the patients and their warfarin dose required to achieve a prothrombin ratio of 1.8 to 2.5 ($r = -0.661$, $P < 0.01$) but not with their prothrombin ratios ($r = 0.110$, $P > 0.05$). Total plasma warfarin clearance varied from 2.56 to 6.37 ml h^{-1} kg^{-1} body weight (mean 4.01 s.d. ± 1.13 ml h^{-1} kg^{-1} body weight) but did not correlate with age ($r = -0.028$, $P > 0.05$).

The free fraction of warfarin varied between 0.0172 and 0.0261 (mean 0.021 s.d. ± 0.003) and correlated with total plasma warfarin clearance ($r = 0.955$, $P > 0.001$: (Figure 1). Free plasma warfarin clearance ranged from 152 to 244 ml h^{-1} kg^{-1} body-weight

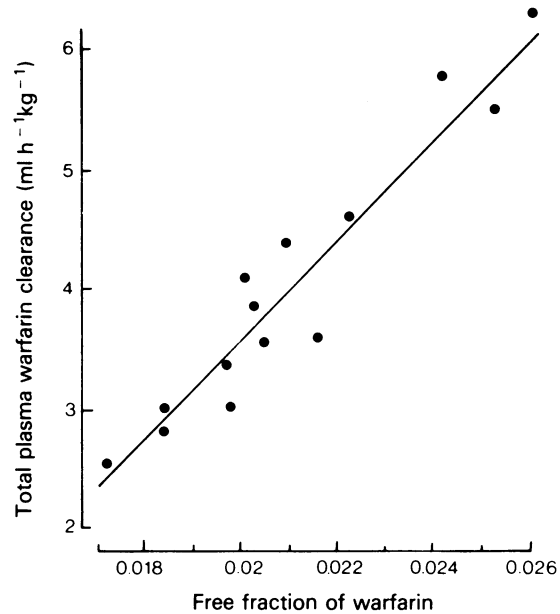

Figure 1 Relationship between total plasma warfarin clearance and the free fraction of warfarin in plasma ($r = 0.955$, $P < 0.001$)

(mean 196 s.d. ± 32 ml h^{-1} kg^{-1} body weight) but was unrelated to age ($r = -0.164$, $P0.05$). The coefficient of variation of free plasma warfarin clearance was 17% compared with 29% for total plasma warfarin clearance. Six of the fifteen patients were cigarette smokers: their mean age of 57.7 years was not significantly different from the mean age of non-smokers (59.0 years), but there was no significant difference in the mean warfarin dose requirements, total and free plasma warfarin clearances, or free warfarin fraction between smokers and non-smokers ($P > 0.05$).

The relationship between the logarithm of the plasma warfarin concentration and the prothrombin ratio for all patients is shown in Figure 2 and appears to be linear above a prothrombin ratio of 1.2.

The steady-state plasma warfarin concentrations (total and free) required to achieve a prothrombin ratio of 1.8 (the lowest prothrombin ratio recorded in any of the patients at the beginning of the study) were calculated from the individual regression equations of the log concentration/effect lines above a prothrombin ratio of 1.2 (Figure 3a & b). The mean total plasma warfarin concentration was 0.67 s.d. ± 0.16 μg/ml and the mean free plasma warfarin concentration was 14.3 s.d. ± 5.2 ng/ml. Both were negatively correlated with age ($r = -0.682$, $P < 0.01$ for both). The slope of the linear portion of the log plasma warfarin concentration-effect curve varied from 20 to

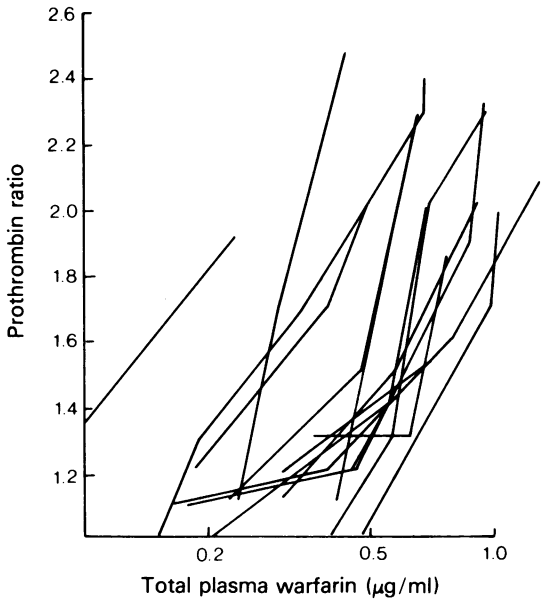

Figure 2 Relationship between the logarithm of the total plasma warfarin concentration and the prothrombin ratio for the 15 patients studied.

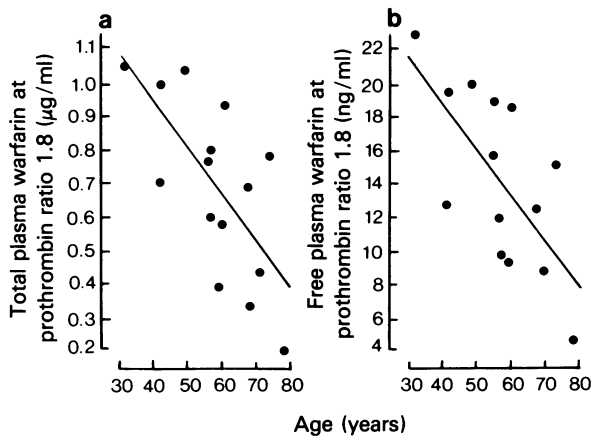

Figure 3 a) Relationship between the total plasma warfarin concentration required to achieve a prothrombin ratio of 1.8 and age ($r=0.0682$, $P<0.01$) b) Relationship between the free plasma warfarin concentration required to achieve a prothrombin ratio of 1.8 and age (0.682, $P<0.01$)

130 (mean 47 s.d. $\pm$34) and was significantly correlated with the free fraction of warfarin ($r=0.586$, $P<0.05$: Figure 4).

Discussion

An age-related decline in the daily dose of warfarin needed to maintain therapeutic anticoagulation amongst 200 patients has been reported previously by us (Routledge, Chapman, Davies & Rawlins, 1979). The similar trend observed in the present study would suggest that the patients are a representative sample of those undergoing treatment with the drug.

Our estimations of the steady-state total plasma warfarin clearance (4.01 s.d. $\pm$ 1.13 ml h^{-1} kg^{-1}) assumes complete compliance and bioavailability (Breckenridge & Orme, 1973), and also that the plasma warfarin concentrations are truly 'mean' steady-state levels (Wagner, Northam, Alway & Carpenter, 1965; Rawlins, Collste, Frisk-Holmberg, Lind, Östman & Sjöqvist, 1974). Since patients were venesected at 22 hours after dosing, we will have underestimated steady-state warfarin concentrations and overestimated clearances. Yacobi, Udall & Levy (1976) using a similar technique to estimate steady-state warfarin clearance measured plasma concentrations at 6 hours after dosing in patients on a 24-hour dosing schedule. This would probably overestimate mean steady-state concentrations and underestimate clearance, and may at least partially explain their lower clearance values (2.53 s.d. $\pm$ 0.92 ml h^{-1} kg^{-1}). Pharmacokinetic theory predicts that if hepatic warfarin extraction is restrictive, the total plasma warfarin clearance should depend on the magnitude of the fraction of warfarin unbound in plasma. This has been substantiated experimentally in both rats (Levy & Yacobi, 1974) and man (Yacobi, Udall & Levy, 1976). In the latter study, the correlation coefficient between total plasma warfarin clearance and free warfarin fraction in 29 patients was $r=0.641$ ($P<0.001$). This indicated that approximately 40% of the variation in total plasma warfarin clearance can be attributed to differences in free fraction of warfarin, a much smaller percentage than in rats. The authors felt that the variation in the relationship was probably due in part to interindividual differences in the activity of warfarin metabolizing enzyme systems, as well as the experimental error in determining the single estimate of total plasma warfarin clearance for each patient. In the present study an average of 4.5 separate estimates of total plasma warfarin clearance were available for each patient, and the correlation coefficient of 0.955 between total plasma clearance and free warfarin fraction indicates that almost 90% of the interindividual variation in clearance is related to differences in protein binding.

Free plasma warfarin clearance is an estimate of the activity of warfarin metabolism. The lack of

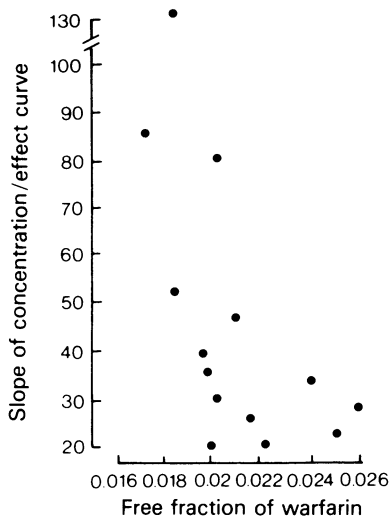

Figure 4 Relationship between the slope of the $\log_{10}$ plasma warfarin concentration/anticoagulant effect curve and the free fraction of warfarin in plasma ($r=0.586$, $P<0.05$)

relationship between this value and the age of the patient indicates that decreasing ability to metabolize racemic warfarin is not responsible for the increased sensitivity to the drug which is seen in the elderly. Similar conclusions have been drawn by Shepherd, Hewick, Moreland and Stevenson (1977) from the results of studies performed with single doses of warfarin.

The absence of significant differences in dosage requirements between smokers and non-smokers accords with the results of Mitchell (1972) which found no difference in dosage requirements between 86 non-smokers, 47 heavy smokers (currently smoking more than 20 cigarettes per day) and 97 light smokers. The observed absence of the effect of smoking habits on free plasma warfarin clearance is in agreement with the findings of Yacobi, *et al.* (1976).

The data shown in Figure 2 demonstrate that there is a clear relationship—within individuals—between the concentration of warfarin in plasma, and its anticoagulant effects as manifested by changes in the prothrombin ratio. Individuals' log concentration-effect curves are linear above a prothrombin ratio of 1.2, and vary widely in both slope and intercept. This variation is reflected in the range of both total (0.19 to 1.03 μg/ml) and free (4.8 to 22.9 ng/ml) plasma concentrations of warfarin which produce the same degree of anticoagulation. Thus, our results are compatible with the observation of Breckenridge & Orme (1973) that plasma warfarin concentrations amongst patients undergoing therapeutic anticoagulation vary widely and suggest that these variations are due to differences between individuals' concentration-effect curves. The fall with age in the free plasma warfarin concentration required to produce a particular anticoagulant effect observed in the present study suggests that the reduced dosage requirements are the result of increased target organ sensitivity. Shepherd *et al.*, (1977) arrived at similar conclusions on the basis of the anticoagulant responses to single doses of warfarin. Although both studies implicate pharmacodynamic factors in the age-related sensitivity to warfarin, they do not completely exclude pharmacokinetic factors. Warfarin is administered as a racemate of R (+) and S (−) warfarin, and the potency of the S (−) enantiomer is approximately five times greater than that of the R (+) enantiomer. The half-life of the S (−) enantiomer is shorter, and its free fraction smaller than the R (+) (Yacobi & Levy, 1977a).

There is, however, marked interindividual variation in the ratio between total plasma R (+) and S (−) clearances, and in eight healthy male volunteers (of unspecified age) the ratio varied from 0.77 to 2.11 (Lewis, Trage, Chan, Breckenridge, Orme, Rowland & Scharry, 1974). Such variation could result in differences in the relative conentration of the enantiomers at steady-state and therefore a difference in the intensity of anticoagulation between patients at the same steady-state plasma concentration of racemic warfarin.

Yacobi & Levy (1977b) recently described a negative relationship between the slope of the log concentration-effect regression line for S (−) warfarin, and its serum-free fraction in rats (Yacobi & Levy, 1977b) both after acute and during chronic dosing. The present study confirms the existence of this relationship in man and suggests that the variation in protein binding may be of importance in reflecting interindividual pharmacodynamic differences. It has been suggested that subjects with steep anticoagulant concentration-effect slopes may be at special risk of excessive or inadequate anticoagulation with only modest increases or decreases in plasma warfarin concentration. Gibaldi & McNamara (1977) have shown in rats that there is a positive correlation between plasma protein binding of warfarin and hepatic tissue binding. It is thus possible that the degree of warfarin binding in plasma reflects the interaction between warfarin and its effector site in the hepatocyte.

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