AGE AS A DETERMINANT OF SENSITIVITY TO WARFARIN

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- 1 Parallel human and rat studies were carried out to confirm the previous suggestion of an increased sensitivity to warfarin in old age.
- 2 The anticoagulant response to warfarin was found to be greater in the elderly groups despite, in the case of the patient study, the elderly subjects being given a smaller weight-related dose.
- 3 At the same plasma warfarin concentrations there was greater inhibition of vitamin K-dependent clotting factor synthesis in the elderly. There was no difference in the rate of clotting factor degradation in the two age groups.
- 4 There was no appreciable difference in warfarin pharmacokinetics (plasma half-life, apparent volume of distribution, plasma clearance, plasma protein binding or plasma warfarin alcohol levels) in the two age groups.
- 5 There appeared to be no major age-related differences in warfarin pharmacokinetics and the increased effect of warfarin in the elderly seemed to result from an increased intrinsic sensitivity to warfarin.

Introduction

On the basis of clinical experience, the indications are that elderly patients are often more sensitive to some drugs than are their young counterparts (Trounce, 1975). This may be a contributory factor in the higher frequency of adverse drug reactions in elderly patients (Hurwitz, 1969).

It has been suggested that elderly patients receiving anticoagulant drugs are more prone to bleeding episodes (Jick, Slone, Borda & Shapiro, 1968; Lamy, 1974; Laurent, 1974) but whether this is due to increased blood vessel fragility or to greater sensitivity to the pharmacological effects of anticoagulant drugs has not been established. In a retrospective epidemiological study O'Malley, Stevenson, Ward, Wood & Crooks (1977) demonstrated that in hospitalized patients over 70 years of age a greater degree of anticoagulation was achieved than in younger patients, despite the former receiving a smaller daily dose of warfarin. This suggested a greater sensitivity to warfarin in the elderly.

The present study was undertaken firstly, to determine whether this apparent age-related increase in sensitivity to warfarin could be demonstrated in a prospective study in man under more precisely defined conditions and secondly, to establish whether the effect could be reproduced in an animal model – the rat. A further objective

was to investigate the mechanism of the altered sensitivity, with particular regard to establishing whether it resulted from differences in warfarin kinetics or in intrinsic sensitivity to warfarin. The results have previously been published in abstract form (Hewick, Moreland, Shepherd & Stevenson, 1975).

Methods

Human studies

None of the patients or volunteers in these studies were receiving drugs known or thought to interfere with warfarin handling or effect, as documented by Hansten (1975). Permission was obtained for all the human studies undertaken from the local ethical committee. All subjects gave informed consent prior to inclusion in the studies.

Anticoagulant response to warfarin in young and elderly patients

(a) *Extent of response following a single dose of warfarin.* Four young (age range 27-37, mean 31 years) and four elderly (age range 62-89, mean 76 years) patients who were to be given oral

anticoagulant therapy were each given a single loading dose of racemic warfarin. The warfarin dose used, which was decided by the clinician in charge, averaged 0.94 mg kg^{-1} in the young patients and 0.55 mg kg^{-1} in the elderly group. Thrombotest activity was determined in approximately ten venous blood samples taken over the next 120 h. In each sample, plasma warfarin and warfarin alcohol concentrations were measured. In all studies, blood was anticoagulated with trisodium citrate (10%, v/v, 0.106 M).

(b) *Rate of clotting factor synthesis and degradation.* To each of the old and young groups described above were added two further patients who were due to discontinue long-term anticoagulant therapy. Warfarin therapy was stopped in these patients and the return to normal Thrombotest activity observed over a period of 96 h. A further warfarin loading dose was then given and the Thrombotest response was monitored over the next 48 h. The results in the additional young and old patients were similar to those observed in the young and old groups described above and the data from all 6 patients in each group were therefore pooled. The warfarin loading dose used averaged 0.96 mg kg^{-1} in young patients (age range 25-37, mean 31 years) and 0.57 mg kg^{-1} in the old patients (age range 62-90, mean 77 years). Approximately ten venous blood samples were taken for the determination of Thrombotest percentage and plasma warfarin and warfarin alcohol concentrations.

Warfarin pharmacokinetics. Thirteen healthy young (age range 20-40, mean 25 years), and thirteen healthy elderly (age range 65-94, mean 82 years) subjects were each given 1.5 mg kg^{-1} of oral racemic warfarin with 50 mg of oral vitamin K_1 and, where necessary, further vitamin K_1 to maintain a normal Thrombotest level. The elderly group of volunteers were ambulant patients in a geriatric unit. Approximately six venous blood samples were taken at intervals for 72 h after warfarin dosing.

The binding of warfarin to plasma proteins *in vitro* was studied by equilibrium dialysis using fresh, undiluted plasma from a further thirteen young (age range 17-37, mean 25 years) and fifteen elderly subjects (age range 68-94, mean 78 years). Plasma (500 μl) was placed inside a dialysis bag made from Visking tubing and the bag sealed and suspended in 8 ml of 0.05 M phosphate buffer (pH 7.4) containing ^{14}C -labelled warfarin (Radiochemical Centre, Amersham) to give a post-dialysis plasma concentration of approximately $2 \mu\text{g ml}^{-1}$. The sealed system was agitated horizontally at 1 Hz in a water bath at 37°C for 5 h in the dark. At the end of this time, equilibrium in the system

had been achieved. Aliquots (50 μl) of both plasma and buffer were then counted in 10 ml of scintillator (Nuclear Enterprises NE 260).

Animal studies

Studies parallel to those described above were carried out using male Wistar rats aged approximately 80 days (young group) and 600 days (old group). Twenty-one rats in each group were injected intraperitoneally with 2.5 mg kg^{-1} of D-warfarin. At intervals up to 36 h, three rats from each group were killed and 5 ml of aortic blood collected from each animal for determination of prothrombin times and plasma warfarin and warfarin alcohol concentrations.

Assay of warfarin and warfarin alcohol

Plasma warfarin and warfarin alcohol concentrations were determined by the method of Lewis, Ilnicki & Carlstrom (1970) as modified by Hewick & McEwen (1973).

The plasma warfarin half-life ($T_{1/2}$) was obtained from a regression line fitted to the logarithm of plasma warfarin concentration versus time curve by the method of least squares. The apparent volume of distribution (V_d) was calculated from the extrapolated zero-time plasma warfarin concentration and the warfarin plasma clearance from the relationship,

$$\text{Clearance} = \frac{0.693 \times V_d}{T_{1/2}}$$

In the second human study, the area under the plasma warfarin alcohol concentration versus time curve was determined using the trapezoidal rule for the first 72 h following warfarin administration.

Measurement of clotting factor degradation and synthesis

Vitamin K-dependent prothrombin complex activity (PCA) was determined in human venous blood using the Thrombotest procedure (B.D.H., Poole, Dorset) and in rat plasma by Quick's (1966) one-stage prothrombin method using Simplastin (Warner-Chilcott Laboratories, N.J., U.S.A.). In the human studies, clotting times were converted to percent normal PCA using calibration curves supplied with each batch of Thrombotest reagent. In the rat studies the conversion was effected using curves constructed by determining the prothrombin times of a series of saline-diluted plasmas from three untreated young and three untreated old rats.

The rates of clotting factor degradation and

Figure 1 The anticoagulant response of (a) young (▲) and elderly (●) patients and (b) young (▲) and old (●) rats to single doses of warfarin. Results are expressed as mean $\pm$ s.e. mean and * represents significant difference between the two age groups in the human study only.

synthesis were determined as follows by the method of Nagashima, O'Reilly & Levy (1969). The rate of degradation (R_{deg}) of PCA is a first order process and may be represented thus:

$$R_{deg} = K_d \times PCA$$

where K_d is the first order rate constant. PCA degradation half-life and K_d were calculated from the initial decline of PCA following a synthesis-blocking dose of warfarin. The rate of synthesis of PCA (R_{syn}) was then calculated for each of the sampling intervals during recovery of PCA, using equation 1 and the relationship,

$$R_{net} = R_{syn} - R_{deg}$$

where R_{net} is the net rate of change in PCA.

Results

Anticoagulant response to warfarin in young and elderly groups

Figure 1 shows the anticoagulant response of four young and four elderly patients and that of young and old rats to single doses of racemic and D-warfarin respectively. PCA was significantly lower in the elderly patients than in the young patients except in the 40-80 and 90-110 h periods (Figure 1a). The duration of response in the two groups of patients was similar despite the doses in the elderly being 41% lower on a weight-related

basis. Equal weight-related doses administered to rats depressed PCA in the old group for approximately 8 h longer than in the young group (Figure 1b).

PCA plasma half-life was 10.8 ± 1.1 h in six young patients and 12.4 ± 1.4 h in six elderly patients (mean $\pm$ s.e. mean). The difference between the means was not significant. The PCA degradation half-lives were identical in young (3.9 h) and old (3.9 h) rats.

To assess the relative sensitivity of clotting factor synthesis to warfarin in the two age groups, the rate of synthesis of clotting factors (R_{syn}) was plotted against the logarithm of plasma warfarin concentration (Figure 2). At the same plasma warfarin concentrations the geriatric groups showed a much greater degree of inhibition of vitamin K - dependent factor synthesis than did the young groups.

Warfarin pharmacokinetics

Table 1 shows warfarin pharmacokinetic data obtained from thirteen young and thirteen elderly volunteers following a single oral dose of racemic warfarin, along with corresponding data from young and old rats injected intraperitoneally with D-warfarin.

Although the mean half-life of warfarin was longer and the plasma clearance slightly lower in the elderly subjects, the differences between the means were not statistically significant. There was no difference between the apparent volumes of distribution in the two age groups. The plasma

Figure 2 For both (a) human and (b) rat studies, the rate of synthesis of clotting factors (R_{Syn}) is plotted against the logarithm of plasma warfarin concentration in the two age groups (● old; ▲ young). Composite regression lines are drawn for each group. Each data point in the rat study represents the mean of three rats.

warfarin half-lives, apparent volumes of distribution and plasma warfarin clearances were similar in young and old rats. There was no significant difference between the slopes of the logarithm of plasma warfarin concentration versus time curves. It is also evident from Table 1 that the extent of binding of warfarin to plasma proteins was identical in the two age groups. The plasma warfarin alcohol levels were similar in the young and elderly patients. No warfarin alcohols were detectable in the plasma from rats of either age group.

Discussion

The retrospective epidemiological study of O'Malley *et al.* (1977) provided evidence of an increased sensitivity to warfarin in the elderly. The results in Figure 1 would seem to confirm this in that firstly, similar hypoprothrombinaemic responses were obtained in young and elderly patients despite the latter group receiving lower weight-related doses and secondly, old rats responded to warfarin for longer than young rats following equal weight-related doses.

Table 1 Warfarin pharmacokinetic data in young and old humans (mean $\pm$ s.e. mean) and rats (mean) following 1.5 mg kg^{-1} oral racemic warfarin and 2.5 mg kg^{-1} intraperitoneal D-warfarin respectively. Warfarin protein binding was measured by a dialysis technique at a final plasma warfarin concentration of $2 \mu\text{g ml}^{-1}$.

	Plasma $T_{1/2}$ (h)	V_d (ml kg^{-1})	Plasma clearance ($\text{ml kg}^{-1} \text{ h}^{-1}$)	Warfarin alcohols – area under plasma concentration curve (0–72 h) ($\mu\text{g h ml}^{-1}$)	% Warfarin bound
Human					
Young	37 ± 2.1	193 ± 26	3.80 ± 0.55	44 ± 6	98.6 ± 0.1
Old	44 ± 3.9	200 ± 12	3.26 ± 0.49	50 ± 7	98.5 ± 0.1
Rat					
Young	5.4	310	39.8	ND	—
Old	5.2	289	38.5	ND	—

ND represents failure to detect warfarin alcohols in rat plasma.

Possible explanations of these findings include an alteration in warfarin pharmacokinetics in the elderly or an increase in their intrinsic sensitivity to warfarin. There is evidence suggesting impaired metabolism as a possible cause, since the rates of elimination of other extensively metabolised drugs such as antipyrine (O'Malley, Crooks, Duke & Stevenson, 1971; Vestal, Norris, Tobin, Cohen, Shock & Andres, 1975), aminopyrine (Jori, di Salle & Quadri, 1972) and paracetamol (Triggs, Nation, Long & Ashley, 1975) have been shown to be lower in the elderly. It has also been demonstrated that elderly subjects convert a smaller proportion of a dose of amylobarbitone sodium to its metabolite 3-hydroxyamylobarbitone, than younger subjects (Irvine, Grove, Toseland & Trounce, 1974). Table 1 indicates however that this is not likely to be a major factor in the increased effect of warfarin in the elderly since there was no statistically significant difference between the mean plasma warfarin clearance in the young and elderly humans and rats. The possibility exists that although the rate of elimination of warfarin is similar in the two age groups, the renal elimination of the alcohols formed (which are known to have some anticoagulant activity) may be reduced in the elderly. However, the warfarin alcohol data in Table 1 indicate that this is not the case. Furthermore, warfarin alcohols were not detectable in plasma from either age group of rats studied.

The lack of change in the apparent volume of distribution of warfarin in both human and animal studies, together with the identical protein binding data in both human age groups (Table 1), would seem to rule out distribution changes being involved. These findings on protein binding are at some variance with those of Hayes, Langman & Short (1975a), who demonstrated a reduction in maximum binding capacity for warfarin in plasma from elderly subjects. In their studies, warfarin was added to the buffer at concentrations from 3-25 $\mu\text{g/ml}$ which would lead to post-dialysis plasma warfarin concentrations some ten-fold higher. This is very much greater than the usual therapeutic plasma warfarin levels (around 2 $\mu\text{g/ml}$), the post-dialysis plasma concentration used in our studies. Moreover, since they diluted the plasma samples 1 in 3 with Tyrode solution,

the warfarin concentration relative to plasma albumin is very much greater than in our study.

With other drugs, the evidence for altered protein binding in old age is also conflicting. The same authors demonstrated a reduced binding of phenytoin in the elderly (Hayes, Langman & Short, 1975b) and the plasma binding of pethidine seems to be reduced in old age (Mather, Tucker, Pflug, Lindop & Wilkenson, 1975). However, no age-related differences in protein binding were reported with penicillin G, phenobarbituric acid and phenytoin by Bender, Post, Meier, Higson & Reichard (1975), nor is there any reduction in the extent of protein binding of diazepam or desmethyldiazepam in elderly subjects (Klotz, Avant, Hoyumpa, Schenker & Wilkinson, 1975).

It appears therefore that no major differences exist in warfarin pharmacokinetics in young and elderly subjects and that the increased effect of warfarin in the elderly results from an increased intrinsic sensitivity to warfarin. Using the method of Nagashima *et al.* (1969) for determination of the rate of clotting factor turnover, no difference was found in the rate of clotting factor degradation in the two age groups. Figure 2 shows clearly that at the same plasma warfarin concentrations, the elderly group show a much greater inhibition of clotting factor synthesis than do their younger counterparts. While it is possible that there is a difference in the ratio of warfarin concentration in liver and plasma in the two age groups, it has been shown (Moreland, 1975), that the liver warfarin concentration closely parallels the plasma concentration in both young and old rats.

The possible reasons for this altered sensitivity of the clotting factor synthesizing system to warfarin include a decreased affinity of the system for vitamin K and an increased affinity for warfarin in the elderly. A further possibility is that elderly subjects are relatively deficient in vitamin K due to reduced dietary intake, defective absorption or alteration in the pharmacokinetics of vitamin K.

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