CHANGES IN DRUG METABOLISM WITH INCREASING AGE: 1 WARFARIN BINDING AND PLASMA PROTEINS

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- 1 A decrease was found in the binding capacity of elderly people for warfarin.
- 2 The decrease in binding correlated with a fall in plasma albumin concentrations.
- 3 No changes in the association constant were observed.

Introduction

The over-reaction or adverse response of the elderly to commonly accepted therapeutic doses of drugs has long been clinically evident (Hurwitz, 1969). Changes in plasma proteins in the aged have also been observed (Woodford-Williams, Alvarez, Webster, Landless & Dixon, 1964) and any such changes in protein levels, particularly of albumin, could have an effect on the handling of highly bound drugs in the elderly. Plasma albumin concentrations have therefore been measured in two groups of people, the first being under 45 years of age and the second over 65 years of age, and detailed studies were also carried out on warfarin binding in sub-groups of both ages.

Methods

The young people were drawn from hospital personnel and the old principally from long-stay geriatric homes in Nottingham. At the time of study, no person was taking any other therapy, all had normal blood urea and serum electrolytes and were clinically in good health. All blood samples were anticoagulated with 3.8% sodium citrate (ratio 9:1 v.v.), the plasma separated by centrifugation and stored at -20°C until used.

Plasma albumin was measured by the H.A.B.A. dye method modified for use on the Technicon autoanalyser (Kness, Dickerson & Pastewka, 1965). Bovine standards and dye were obtained from Sigma Chemicals Ltd. All measurements were carried out in triplicate, together with blank estimates. Standards were run with each batch.

Binding studies were carried out by equilibrium dialysis (O'Reilly & Kowitz, 1967). Plasma samples were diluted 1:3 with calcium free Tyrode solution (Tyrode, 1910) and 1 ml aliquots dialysed against 10 ml of the same Tyrode

solution, to which warfarin containing a known quantity of ^{14}C -labelled drug was added. Triplicate samples of the test plasma were exposed to four different concentrations (from 9 to $80\text{ }\mu\text{mol/l}$) of warfarin and dialysed for 16-18 h at 37°C in Visking sacs by which time equilibrium had been reached. Controls containing warfarin-Tyrode solution outside the sac and Tyrode solution inside the sac were included in all experiments and showed that equilibrium was achieved in this period of time. Separate experiments showed that the use of citrate did not affect binding.

Aliquot samples of the plasma and the Tyrode solution were assayed for ^{14}C -activity after the completion of dialysis, using a Beckman LS-100 Liquid Scintillation Counter. Free warfarin levels were measured as the concentrations remaining in the Tyrode solution. Bound concentrations were calculated by subtraction of the free concentration from the total concentration measured in the plasma. Drug binding to the Visking membrane was of the order of 3%, but measurement of the drug levels on both sides of the membrane obviated the need for consideration of the membrane binding of the drug when calculating the results.

Any protein leakage through the sacs was detected by analysing a sample of the post-dialysis Tyrode solution using the Folin-phenol method adapted for use on the autoanalyser (Lowry, Rosebrough, Farr & Randall, 1951). Contaminated samples were discarded.

Results

Dilution of the plasma reduced the calculated error from the Donnan effect to 0.9%, assuming a maximum plasma protein anion concentration of

Figure 1 A typical Rosenthal plot for warfarin binding.

16 mEq/l (Gamble, 1954) and so no correction was applied to the results obtained

Analysis of the binding data was carried out using the Rosenthal equation (Rosenthal, 1967). This can be expressed in the form:

$$\frac{r}{D} = NK - rK$$

where r = moles of drug bound
 R = free drug concentration
 K = association constant
 N = number of binding sites.

Analysis of this equation allows the maximum number of binding sites/unit of plasma to be calculated, together with the association constant. At the drug concentrations used in this study calculation of the binding results using the

Figure 2 Comparison of plasma albumin concentration between two age groups. The vertical bar indicates the mean $\pm$ 2 s.e. mean. The difference between the two groups is significant ($P < 0.001$).

Table 1 Maximum plasma binding of warfarin in two age groups

Subject	Age (years)	Maximum warfarin binding ($\mu\text{mol/l}$)	Subject	Age (years)	Maximum warfarin binding ($\mu\text{mol/l}$)
1	20	624	10	65	424
2	21	480	11	70	384
3	22	624	12	73	524
4	23	532	13	76	504
5	23	588	14	79	524
6	30	548	15	79	600
7	32	560	16	80	388
8	42	544	17	84	384
9	45	548	18	86	420
			19	86	328
			20	87	472
			21	90	460

$$\bar{x} = 561 \pm 15$$

$$t = 3.7783$$

$$P < 0.002$$

$$\bar{x} = 451 \pm 22$$

Rosenthal equation indicated a single class of binding site. A typical plot is shown in Figure 1. Table 1 shows a fall in the maximum binding of warfarin to this site/litre of plasma in the elderly group and this was highly significant statistically. Analysis of the values found for the association constant showed no differences between the two groups and the mean value obtained was 24.5×10^{-4} M (Table 2).

Comparison of the levels of plasma albumin showed a reduction in the group over 65 years of age (Figure 2). The mean levels found were 3.9 g% and 3.0 g% in the younger and older group respectively and this difference was statistically significant ($P < 0.001$). Further analysis showed this difference to be present when comparing young and old male and female groups separately. There was also a significant difference in protein levels between the male and female persons over the age of 65 years. No such difference was observed between the young males and females. A strong correlation was found between the maximum binding sites and the plasma albumin concentration (Figure 3).

Discussion

Changes in plasma protein concentration with age have been previously described (Woodford-Williams *et al.*, 1964; Haferkamp, Schlettwein-Gsell, Schwick & Storiko, 1966; Rafsky, Brill, Stern & Corey, 1952). Though Parfentjev & Johnson (1955) have detected no change, the pattern generally found has been a fall in albumin level with a rise in gamma globulin concentrations

Figure 3 Regression of maximum plasma warfarin binding sites against plasma albumin ($r = 0.698$, d.f. = 19 and $P < 0.001$).

as age increases. No authors found great differences between the sexes. The most important cause for these changes seems to be related to a decreased mobility of old people resulting from a variety of causes (Woodford-Williams *et al.*, 1964). The fall in plasma albumin concentration is seen in the present series which represents a broad range of elderly patients. Any such reduction in plasma albumin level could lead to alteration in drug handling in the elderly.

In vivo warfarin is solely bound to albumin (O'Reilly & Kowitz, 1967). We have clearly shown a reduction in the binding capacity of warfarin in the elderly. Almost certainly this is due to the fall in plasma albumin since the binding sites/litre of

Table 2 Warfarin association constant and age

Subject	Age (years)	Association constant $\times 10^{-4}$ M ₁	Subject	Age (years)	Association constant $\times 10^{-4}$ M ₁
1	20	20.4	10	65	31.4
2	21	30.6	11	70	20.5
3	22	21.3	12	73	26.8
4	23	27.2	13	76	20.0
5	23	20.3	14	79	26.3
6	30	24.8	15	79	21.2
7	32	23.5	16	80	24.8
8	42	27.5	17	84	24.5
9	45	20.4	18	86	19.6
			19	86	34.2
			20	87	22.1
			21	90	26.3

$$\bar{x} = 24.0 \pm 1.3$$

$$t = 0.4313$$

$$P > 0.05$$

$$\bar{x} = 24.8 \pm 1.3$$

plasma correlated so closely to the albumin levels.

This finding should be interpreted with some care as the estimation of plasma albumin in this study uses dye binding to albumin. Thus the strict conclusion should be that the ratio (H.A.B.A. sites/warfarin sites) is unchanged. However, the reduction in warfarin binding capacity has been demonstrated independently of the albumin values obtained by the H.A.B.A. method and such albumin determinations could be used as a useful indicator of drug binding ability.

Calculation of the affinity constant again independently of albumin shows no change with age and the mean value of 24.5×10^{-4} compares with the value of 23×10^{-4} M found by O'Reilly (1969). It would seem that the strength of warfarin binding is uninfluenced by age.

The effect of reduced binding would be to allow more free drug to be available at any moment for distribution to other body tissues.

This would at least transiently enhance the effect of a drug, whether it is active before or after metabolic transformation.

It is clearly probable not only that the main effects of protein bound drugs will be affected by changes in binding site concentration, but that the incidence of side effects is also likely to change. It has previously been shown, not only that elderly people are more prone to side effects of drugs (Hurwitz, 1969), but that an increased incidence of side effects in the elderly can occur following anticoagulation (Peyman, 1958). At least part of such an effect could be due to the reduced drug binding capacity which we have found.

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