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FACTORS AFFECTING DRUG BINDING IN PLASMA OF ELDERLY PATIENTS

SUSAN WALLACE & B. WHITING

Department of Materia Medica, University of Glasgow, Stobhill General Hospital, Glasgow G21 3UW

J. RUNCIE

Department of Geriatric Medicine, University of Glasgow, Stobhill General Hospital, Glasgow G21 3UW

1 The binding of salicylate, sulphadiazine and phenylbutazone to plasma proteins has been studied in young and elderly subjects.

2 Elderly patients had significantly reduced concentrations of plasma albumin, compared with subjects under 40 years of age.

3 Significant increases in free levels of all three drugs were found in elderly patients receiving multiple drug therapy, and a correlation obtained with the number of drugs being taken.

4 It is suggested that because of their low albumin levels, elderly patients may be more susceptible to the effects of multiple drug therapy on drug binding. The clinical implications of these observations are discussed.

Introduction

It has been established that the elderly are particularly prone to drug toxicity (Hurwitz, 1969). This implies that there may be some alteration in drug handling with ageing. Convincing evidence of this was lacking until O'Malley, Crooks, Duke & Stevenson (1971) found that the half-lives of antipyrine and phenylbutazone were prolonged in a group of geriatric patients. More recently, Hayes, Langman & Short (1975b) found that phenytoin clearance was increased in an elderly group. This correlated inversely with reductions in both phenytoin binding and plasma albumin levels. Changes in plasma protein concentrations with age had previously been noted by Woodford-Williams, Alvarez, Webster, Landless & Dixon (1964). A reduction in warfarin protein binding in elderly people (Hayes, Longman & Short, 1975a) was attributed partly to reduced albumin levels.

We have been interested in the various factors which may be responsible for changes in protein binding in the elderly and have assessed the contribution made by both decreased albumin levels and multiple drug therapy.

Methods

Plasma protein binding studies were performed on four groups of subjects.

A. Sixteen healthy volunteers, taking no drugs, aged 19-40 years, mean 27 years.
B. Fifteen surgical and gynaecological patients, aged 14-39 years, mean 30 years, not acutely ill, but taking sedatives, analgesics and a variety of other drugs including antibiotics.
C. Sixteen elderly patients taking no drugs, aged 69-85 years, mean 79 years.
D. Twenty-two elderly patients, aged 74-92 years, mean 84 years, taking one or more drugs.

Blood samples (10 ml) were withdrawn from hospital patients approximately 2 h after the first morning drug administration round, and collected into tubes containing lithium-heparin as anticoagulant. The samples were immediately separated by centrifugation. An aliquot of plasma was reserved for routine biochemical tests and protein assay, and the rest was stored at -20° C until use. Albumin was measured on the Technicon Autoanalyzer, using the bromcresol green method.

Protein binding studies were carried out by ultrafiltration, using Amicon Centriflo membrane cones and the Amicon multi-micro ultrafiltration system (MMC). Partition of the free from the protein bound drug was achieved by centrifugation with the Centriflo cones and gas pressure (nitrogen at 30 psi) with the MMC apparatus.

Aliquots of plasma (1 ml) were incubated for

30 min at room temperature (22° C) with salicylic acid (salicylate) (280-400 μg/ml), sulphadiazine (300-500 μg/ml) and phenylbutazone (75-200 μg/ml). Ultrafiltration was then carried out, and samples of the original plasma-drug solution and ultrafiltrate were analysed for total and free drug. Salicylate was determined by the method of Trinder (1954), sulphadiazine by the method of Bratton & Marshall (1939) and phenylbutazone by the method of Andréason (1973). Correction was made for non-specific binding of the drugs to the ultrafiltration membrane, and sulphosalicylic acid was routinely used to check for protein leakage. Any contaminated ultrafiltrates were discarded.

A separate series of experiments was performed to determine the effect of plasma dilution on the binding of the three test drugs. Serial dilutions of 1:5, 1:20 and 1:100 were made of normal plasma using Sørensen's phosphate buffer, pH 7.4. The three drugs were added as previously described to these solutions and ultrafiltration carried out as before.

Results

Percentage free drug for all three drugs in each group of subjects is shown in Figure 1. The elderly patients receiving drugs (Group D) showed a significant increase in percentage free salicylate, sulphadiazine and phenylbutazone when compared to Group A $P < 0.005$ for salicylate; $P < 0.01$ for sulphadiazine; and $P < 0.05$ for phenylbutazone). Groups B and C, young patients receiving drugs and elderly patients not on drug therapy, showed significant increases in percentage free phenylbutazone ($P < 0.05$).

Comparison of the plasma albumin levels in each group showed a significant reduction ($P < 0.001$) in subjects over 70 years of age, i.e. in groups C and D (Figure 2). The levels in each group were: Group A 42 ± 3 g/litre; Group B 39 ± 5 g/litre Group C 36 ± 5 g/litre; and Group D 36 ± 3 g/litre.

Figure 3 shows the effect of *in vitro* plasma dilution on the binding of all three drugs. For both salicylate and sulphadiazine, a considerable reduction (1:20 or greater) in plasma protein concentration would be necessary before a significant (two-fold) increase in unbound drug occurred. For phenylbutazone, however, quite small reductions in plasma concentration, of the order of 1:2 or less, may significantly increase the amount of free drug.

In Figure 4, the effect of number of drugs being taken on binding of salicylate is illustrated for Groups C and D. There is a highly significant increase in free salicylate ($P < 0.001$) when two or more drugs are being taken simultaneously. A similar result was observed with sulphadiazine.

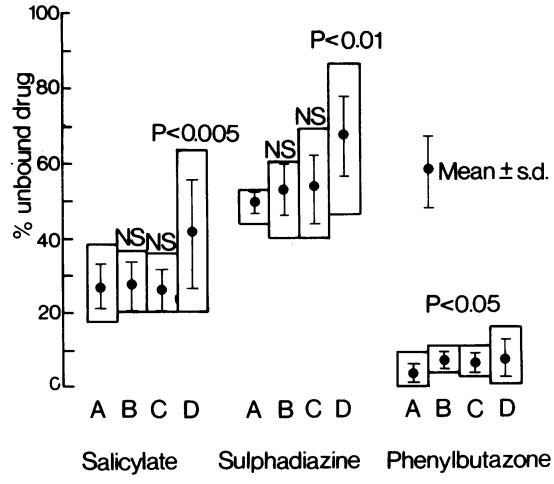

Figure 1 Binding of salicylate, sulphadiazine and phenylbutazone in groups A, B, C and D. Group A: normal young volunteers receiving no drugs. Group B: young surgical and gynaecological patients receiving drug therapy. Group C: elderly patients receiving no drugs. Group D: elderly patients receiving drug therapy. Groups B, C and D are compared to Group A.

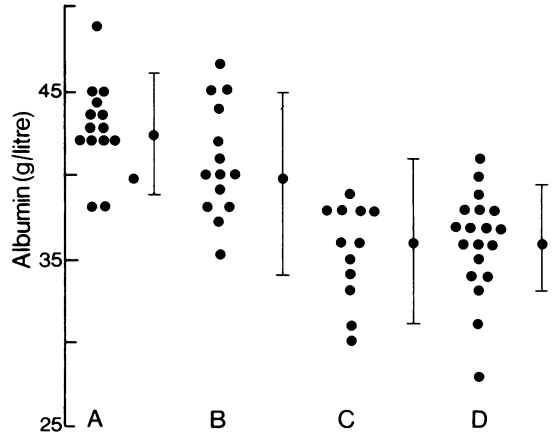

Figure 2 Comparison of plasma albumin concentrations between groups A, B, C and D. The vertical bar indicates the mean ± s.d. Groups C and D are significantly different ($P < 0.001$) from Group A.

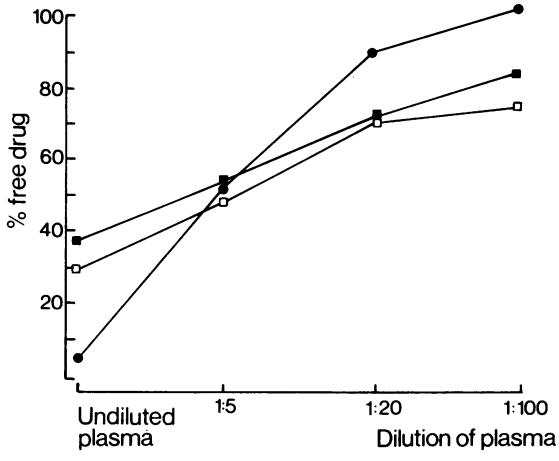

Figure 3 The effect of dilution on the plasma protein binding of salicylate (□ 400 μg/ml), sulphadiazine (■ 500 μg/ml) and phenylbutazone (● 250 μg/ml).

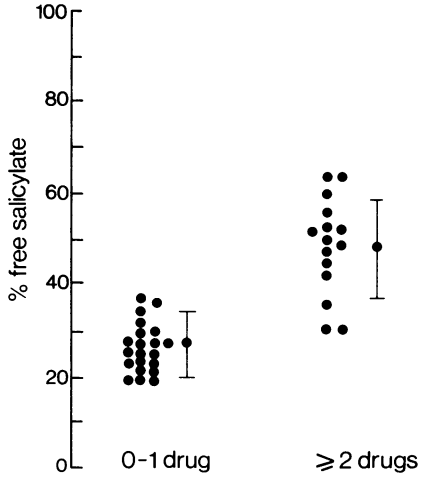

Figure 4 The effect of number of drugs being taken simultaneously on the binding of salicylate in Group C and D subjects. The difference between the two groups is significant ($P < 0.001$). Vertical bars indicate the mean ± s.d.

Discussion

With advancing age, a variety of factors operate to alter the response of a patient to a drug. Changes in drug metabolism (O'Malley *et al.*, 1971), warfarin binding (Hayes *et al.*, 1975a) and phenytoin clearance (Hayes *et al.*, 1975b) have been reported. The present study has concentrated on the effects of reduced albumin levels and multiple drug therapy on the protein binding of phenylbutazone, a highly protein bound drug, and two drugs bound to a lesser extent, salicylate and sulphadiazine.

Changes in plasma protein concentrations with age have been previously described (Woodford-Williams *et al.*, 1964; Hayes *et al.*, 1975a & b). The pattern generally found has been a fall in albumin level with a rise in gamma globulin concentrations as age increases. The most important cause for these changes appears to be related to decreased mobility in elderly people (Woodford-Williams *et al.*, 1964). Our results confirm a fall in plasma albumin in patients over 70 years of age, and this reduction in albumin level could lead to altered drug handling in these patients.

In this study we have found that the binding of phenylbutazone is significantly reduced in elderly patients. From the results of our plasma dilution experiments it can be seen that phenylbutazone binding appears to be particularly affected by alterations in albumin concentration. Neither of the two less extensively bound drugs, salicylate and sulphadiazine showed a significant reduction in binding in elderly patients not receiving drugs, and plasma dilution curves indicate that changes in albumin levels do not have such a pronounced effect on the binding of these drugs.

The incidence of toxic effects increases dramatically with the number of drugs which patients take simultaneously (Smith, Seidl & Cluff, 1966). Displacement of a drug from plasma proteins by competition from other drugs for the same binding sites may have a profound effect on the distribution of that drug. Anton & Corey (1971) have shown that competition may be enhanced when the plasma albumin concentration is reduced. We have studied both young and elderly patients receiving a variety of drugs. Binding of salicylate, sulphadiazine and phenylbutazone was significantly lower in elderly patients receiving drug therapy, compared to normal healthy young adults and also to elderly people not receiving drugs. Only phenylbutazone binding was significantly reduced in young patients receiving drug therapy; the mean albumin level of this group was not significantly different from that of healthy young adults.

It appears, therefore, that the combination of low albumin levels and multiple drug therapy may be responsible for the observed reduction in binding of the three test drugs in elderly patients receiving drug therapy. Of the three test drugs, salicylate binding was reduced most in elderly patients on multiple drug therapy. The precise

reason for this is not known, but may reflect differences in the strength of binding of the drugs. In addition, low albumin concentration alone can cause significant increases in the amount of free drug available for phenylbutazone and other highly protein bound drugs (Hayes *et al.*, 1975 a & b).

The group of young patients receiving multiple drug therapy had albumin levels within the normal range, and only phenylbutazone binding was significantly reduced. Perhaps because of their higher plasma albumin concentrations, this group did not seem as susceptible to the effects of multiple drug therapy on protein binding as did elderly patients.

The effect of reduced binding would be to allow more free drug to become available for distribution throughout the body tissues, thus enhancing, at least transiently, the effect of that drug, or causing more drug to become available for metabolism. This is of particular importance for highly bound drugs (90% or more); the effects for less extensively bound drugs such as sulphadiazine or salicylate would be less marked. It should be borne in mind, however, that the use of plasma drug levels in the design of individual dosage schedules should take into account both free and bound concentrations of the drug, as inter-individual differences in drug binding could be of importance. If elderly patients must be given more than one drug at a time, and if any of these are known to be highly protein bound, it may be advisable to test each individual's plasma for its ability to bind the drug in question.

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Reprint requests should be addressed to B.W.

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