*Br. J. clin. Pharmac.* (1975), 2, 73-79

# CHANGES IN DRUG METABOLISM WITH INCREASING AGE: 2. PHENYTOIN CLEARANCE AND PROTEIN BINDING

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1 Comparison of phenytoin clearance showed a marked increase in people over 65 years of age compared with people under 45 years of age.

2 This difference was apparent if phenytoin was given orally or intravenously.

3 Phenytoin clearance correlated inversly with phenytoin binding and plasma albumin, both of which were found to be reduced in the elderly.

## Introduction

Little information is available about the changes which occur in the metabolic handling of commonly used drugs as age increases, though the frequency with which adverse effects are recognised in the elderly argues that capacity for drug metabolism is altered. We have studied phenytoin clearance. Phenytoin is metabolised by hepatic microsomal enzymes (Maynert, 1960) and the rate of this metabolism may be altered by previous treatment with inducing agents. Phenytoin is safe to administer and easy to measure in plasma.

## Methods

Phenytoin was given in a single oral or intravenous dose, with or without phenobarbitone pretreatment (60 mg daily for three days), to volunteers to whom the procedure had been explained. A younger group of subjects was drawn principally from medical and nursing personnel and an older group from the long stay Geriatric Homes in Nottingham. All were apparently in good health at the time of the study and all had normal blood urea and serum electrolyte concentrations. No person was receiving any therapy at the time of the study nor had done so within the previous eight weeks.

The design of the study is shown in Table 1. Prior to administration of the drugs a sample of blood was drawn for protein measurement and binding studies. Five to six 10 ml blood samples were taken at intervals between 8-36 h following phenytoin administration. All samples were citrated with 3.8% sodium citrate (9 : 1 v/v), the plasma separated and stored at $-20^{\circ}$C until the measurements were carried out.

Phenytoin (Sigma Chemicals Ltd.) was measured by g.l.c. using a flash methylation technique (MacGee, 1970) on a Varian Autograph 705 single column machine with a flame ionisation detector. All determinations were carried out in triplicate and controls and standards were run on each day of the estimations. Drug concentrations were calculated by comparing the ratio of the peak height of phenytoin over an internal standard 5-(*p*-methyl phenyl)-5-phenylhydantoin (Aldrich Chemical Co. Inc.) against a standard curve. The coefficient of variation for replicate samples ranged from 5.9 ± 3.4% at a phenytoin concentration of 10 μg/ml to 7.2 ± 3.4% at 1 μg/ml.

Plasma albumin was calculated by the H.A.B.A. dye (Sigma Chemicals Ltd.) method modified for use on a Technicon autoanalyser (Kness, Dickerson & Pastewka, 1965). Bovine albumin standards (Sigma Chemicals Ltd.) were used. All measurements were carried out in duplicate and with blank estimates. The plasma albumin levels

**Table 1** Subjects and therapy groups

| | *Treatments* | *(a) < 45 years* | *(b) > 65 years* |
|---|---|---|---|
| 1 | Oral phenytoin (500 mg) | Total 10<br>Female 2 | Total 11<br>Female 9 |
| 2 | i.v. phenytoin (250 mg) | Total 10<br>Female 5 | Total 10<br>Female 8 |
| 3 | i.v. phenytoin (250 mg) after phenobarbitone pre-treatment (60 mg daily for 3 days) | Total 11<br>Female 5 | Total 9<br>Female 7 |

were measured in a single batch in each of the three studies.

Binding studies were carried out by equilibrium dialysis (O'Reilly & Kowitz, 1967) in Visking sacs against calcium-free Tyrode solution (Tyrode, 1910). Unlike the warfarin study, phenytoin binding was measured using undiluted plasma. Dilution of plasma has been observed to interfere with phenytoin binding (Lunde, Rane & Yaffee, 1970). The labelled drug was added to the Tyrode solution. Dialysis was carried out for 16-18 h at 4° C. Controls containing phenytoin-Tyrode solution outside the sac and Tyrode solution inside were included in all experiments and showed that equilibrium was achieved in this time. Aliquot samples of plasma and outer Tyrode solution were then counted for $^{14}C$ activity in a Beckmann Liquid Scintillation counter to obtain total and free drug concentrations.

Duplicate samples of plasma were exposed to four concentrations of phenytoin (15, 30, 170 and 300 μmol/l). A check for protein leakage through the Visking sac was made by analysing a sample of the post-dialysis Tyrode solution for protein using the Folin-phenol method adapted for use on an autoanalyser (Lowry, Rosebrough, Farr and Randall, 1951).

### *Calculation of Results*

(a) *Phenytoin clearance* Over the time course studied and at the plasma drug levels achieved, plasma phenytoin followed an exponential curve which can be expressed in the form

$$Cn_t = Cn_o \cdot e^{-kt}$$

where t = time
$Cn_t$ = phenytoin concentration at t
$Cn_o$ = phenytoin concentration at t = 0
k = logarithmic slope of the line (decay constant

The linear regression of log phenytoin on time was calculated and the values of $Cn_0$ and k obtained. Clearance is then given by the formula

$$\text{Clearance} = \frac{\text{Dose of drug administered}}{Cn_o} \times k$$

The result was corrected on a body weight basis since body fluid compartment volumes are related to body size (Haist, 1961).

(b) *Protein binding* The measurements of free and bound drug at the four different drug concentrations were examined by the Rosenthal equation (Rosenthal, 1967). As with the warfarin study the maximum quantity of phenytoin bound (μmol/l of plasma) was calculated together with the association constant. Using undiluted plasma the Donnan effect was estimated to produce an excess of up to 8.3% in the free drug concentration outside the sac over that present within the sac on the basis of a protein anion concentration of 16 mEq/l (Gamble, 1954). Since this would have a greater effect at higher protein concentrations and so accentuate the apparent phenytoin binding differences found in the plasma of different protein content, no 'correction' was attempted.

## Results

Table 2 shows the individual clearance results obtained following the oral administration of

**Table 2** Comparison of phenytoin clearance in two age groups given oral phenytoin (500 mg)

| *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* | *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* |
|---|---|---|---|---|---|
| 1 | 20 | 22.0 | 11 | 67 | 29.0 |
| 2 | 24 | 19.7 | 12 | 68 | 32.8 |
| 3 | 26 | 30.3 | 13 | 70 | 34.8 |
| 4 | 27 | 45.2 | 14 | 71 | 35.1 |
| 5 | 27 | 37.8 | 15 | 72 | 25.6 |
| 6 | 29 | 10.9 | 16 | 79 | 45.6 |
| 7 | 30 | 28.7 | 17 | 80 | 46.3 |
| 8 | 30 | 10.7 | 18 | 85 | 51.2 |
| 9 | 32 | 17.6 | 19 | 91 | 21.2 |
| 10 | 43 | 37.9 | 20 | 93 | 56.0 |
| | | | 21 | 95 | 87.1 |
| | $\bar{x}$ = 26.1 ± 3.7 | | | $\bar{x}$ = 42.2 ± 5.6 | |

$t = 2.3614$
$P < 0.05$

phenytoin without phenobarbitone pre-treatment in the younger (1a) and the older group (1b). Clearance was clearly and consistently greater in the older group and the difference was statistically significant ($P < 0.05$).

Similar results were obtained when the drug was given intravenously and the figures are shown in Table 3 ($P < 0.05$).

The phenobarbitone pre-treated elderly group 3b had an apparently greater phenytoin clearance than group 2b who had not received such pre-treatment (Table 4). This difference, however, fails to reach significant levels ($P > 0.05$). Phenytoin clearance, though, in the pre-treated group remained greater in the elderly than the young.

As no consistent induction effect was observed the results of the two intravenous groups (2 and 3) were pooled in order to increase the number of studies for further analysis. Table 5 shows the comparison of clearance in the combined groups.

Table 6 illustrates the difference between the two age groups when only women are considered ($P < 0.01$). There were insufficient elderly men to analyse the changes in them.

Maximum phenytoin binding concentrations were calculated in 35 of the 39 individuals in the combined group. In four instances samples were inadequate for the measurements to be carried out. Table 7 shows that the maximum binding capacity fell with age and the change was highly significant statistically ($P < 0.001$). The reduction

**Table 3** Comparison of clearance in two age groups given i.v. phenytoin (250 mg)

| *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* | *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* |
|---|---|---|---|---|---|
| 22 | 20 | 29.6 | 32 | 65 | 78.6 |
| 23 | 20 | 45.1 | 33 | 75 | 35.3 |
| 24 | 25 | 55.1 | 34 | 75 | 59.8 |
| 25 | 27 | 42.9 | 35 | 79 | 74.1 |
| 26 | 27 | 43.5 | 36 | 80 | 113.5 |
| 27 | 30 | 59.0 | 37 | 80 | 92.6 |
| 28 | 30 | 27.3 | 38 | 81 | 35.8 |
| 29 | 34 | 62.5 | 39 | 83 | 36.7 |
| 30 | 35 | 45.0 | 40 | 83 | 93.4 |
| 31 | 38 | 32.5 | 41 | 86 | 53.3 |
| | $\bar{x} = 44.3 \pm 3.8$ | | | $\bar{x} = 67.3 \pm 8.7$ | |

$t = 2.4237$
$P < 0.05$

**Table 4** Comparison of phenytoin clearance (ml kg$^{-1}$ h$^{-1}$) results of induced (phenobarbitone, 60 mg daily for 3 days) and non-induced groups given i.v. phenytoin (250 mg)

| *(a)* | *Non-induced Group* | *Induced Group* |
|---|---|---|
| < 45 years | $\bar{x} = 44.3 \pm 12.1$ | $\bar{x} = 42.0 \pm 18.5$ |
| > 65 years | $\bar{x} = 67.3 \pm 23.8$ | $\bar{x} = 73.2 \pm 18.6$ |
| Combined groups < 45 and > 65 years | $\bar{x} = 55.8 \pm 23.8$ | $\bar{x} = 56.1 \pm 24.1$ |

*(b)* *Variance ratio of non-induced to induced groups*

1. < 45 years group $= \frac{340.845}{145.781} = 2.338$ $P > 0.05$
2. > 65 years group $= \frac{759.388}{347.115} = 2.188$ $P > 0.05$
3. Combined group < 45 and > 65 years $= \frac{579.076}{568.679} = 1.016$ $P > 0.05$

**Table 5** Comparison of two age groups given i.v. phenytoin (250 mg) with and without phenobarbitone (60 mg daily for 3 days) induction

| *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* | *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* |
|---|---|---|---|---|---|
| 42 | 20 | 62.6 | 32 | 65 | 78.6 |
| 22 | 20 | 29.6 | 53 | 66 | 82.3 |
| 43 | 20 | 43.5 | 54 | 72 | 65.8 |
| 23 | 20 | 45.1 | 55 | 72 | 58.8 |
| 44 | 22 | 26.6 | 56 | 75 | 57.4 |
| 45 | 24 | 27.0 | 33 | 75 | 35.3 |
| 24 | 25 | 55.1 | 34 | 75 | 59.8 |
| 46 | 26 | 43.8 | 57 | 79 | 77.5 |
| 25 | 27 | 42.9 | 35 | 79 | 74.1 |
| 26 | 27 | 43.5 | 36 | 80 | 113.5 |
| 47 | 27 | 60.4 | 37 | 80 | 92.6 |
| 48 | 29 | 29.4 | 38 | 81 | 35.8 |
| 27 | 30 | 59.0 | 39 | 83 | 36.7 |
| 28 | 30 | 27.3 | 40 | 83 | 93.4 |
| 49 | 30 | 15.8 | 58 | 85 | 56.2 |
| 50 | 30 | 68.5 | 41 | 86 | 53.3 |
| 51 | 32 | 60.1 | 59 | 87 | 77.5 |
| 52 | 32 | 24.6 | 60 | 89 | 67.7 |
| 29 | 34 | 62.5 | 61 | 90 | 115.9 |
| 30 | 35 | 45.0 | | | |
| 31 | 38 | 32.5 | | | |
| | $\bar{x}$ = 43.1 ± 3.4 | | | $\bar{x}$ = 70.1 ± 5.3 | |

$t$ = 4.3671
$P < 0.001$

**Table 6** Comparison of two female age groups given i.v. phenytoin (250 mg)

| *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* | *Subject* | *Age (years)* | *Clearance (ml kg$^{-1}$ h$^{-1}$)* |
|---|---|---|---|---|---|
| 22 | 20 | 29.6 | 53 | 66 | 82.3 |
| 23 | 20 | 45.1 | 55 | 72 | 58.8 |
| 42 | 20 | 62.6 | 56 | 75 | 57.4 |
| 43 | 20 | 43.5 | 34 | 75 | 59.8 |
| 44 | 22 | 26.6 | 35 | 79 | 74.1 |
| 24 | 25 | 55.1 | 36 | 80 | 113.5 |
| 47 | 27 | 43.8 | 37 | 80 | 92.6 |
| 51 | 32 | 60.1 | 38 | 81 | 35.8 |
| 29 | 34 | 62.5 | 39 | 83 | 36.7 |
| 30 | 35 | 45.0 | 40 | 83 | 93.4 |
| | | | 58 | 85 | 56.2 |
| | | | 41 | 86 | 53.3 |
| | | | 59 | 87 | 77.5 |
| | | | 60 | 89 | 67.7 |
| | | | 61 | 90 | 115.9 |
| | $\bar{x}$ = 47.4 ± 4.0 | | | $\bar{x}$ = 71.7 ± 6.3 | |

$t$ = 2.8735
$P < 0.01$

**Table 7** Maximum plasma binding of phenytoin with age

| *Subject* | *Age (years)* | *Maximum phenytoin binding (μmol/l)* | *Subject* | *Age (years)* | *Maximum phenytoin binding (μmol/l)* |
|---|---|---|---|---|---|
| 22 | 20 | 903 | 32 | 65 | 626 |
| 23 | 20 | 698 | 53 | 66 | 626 |
| 44 | 22 | 826 | 54 | 72 | 769 |
| 45 | 24 | 933 | 55 | 72 | 713 |
| 24 | 25 | 733 | 33 | 75 | 510 |
| 46 | 26 | 650 | 34 | 75 | 592 |
| 25 | 27 | 638 | 56 | 75 | 625 |
| 26 | 27 | 693 | 35 | 79 | 604 |
| 48 | 29 | 686 | 57 | 79 | 669 |
| 27 | 30 | 664 | 36 | 80 | 430 |
| 49 | 30 | 669 | 37 | 80 | 530 |
| 28 | 30 | 691 | 38 | 81 | 572 |
| 51 | 32 | 656 | 39 | 83 | 552 |
| 52 | 32 | 818 | 40 | 83 | 675 |
| 29 | 34 | 768 | 41 | 86 | 667 |
| 30 | 35 | 614 | 58 | 85 | 508 |
| 31 | 38 | 720 | 59 | 87 | 629 |
| | | | 60 | 89 | 398 |
| | | | 61 | 90 | 610 |
| | $\bar{x}$ = 727 ± 22 | | | $\bar{x}$ = 595 ± 21 | |

$t = 4.2815$
$P < 0.001$

**Table 8** Maximum plasma binding of phenytoin with plasma albumin

| *Subject* | *Albumin (g/l)* | *Maximum phenytoin binding (μmol/l)* | *Subject* | *Albumin (g/l)* | *Maximum phenytoin binding (μmol/l)* |
|---|---|---|---|---|---|
| 22 | 38 | 903 | 32 | 32 | 626 |
| 23 | 42 | 698 | 53 | 32 | 626 |
| 44 | 45 | 826 | 54 | 27 | 769 |
| 45 | 42 | 933 | 55 | 29 | 713 |
| 24 | 38 | 733 | 33 | 29 | 510 |
| 46 | 41 | 650 | 34 | 25 | 592 |
| 25 | 42 | 638 | 56 | 29 | 625 |
| 26 | 38 | 693 | 35 | 30 | 604 |
| 48 | 42 | 686 | 57 | 30 | 669 |
| 27 | 41 | 664 | 36 | 23 | 430 |
| 49 | 42 | 669 | 37 | 26 | 530 |
| 28 | 39 | 691 | 38 | 26 | 572 |
| 51 | 43 | 658 | 39 | 29 | 675 |
| 52 | 44 | 818 | 41 | 30 | 667 |
| 29 | 34 | 768 | 58 | 31 | 508 |
| 30 | 41 | 614 | 59 | 29 | 629 |
| 31 | 38 | 720 | 60 | 26 | 398 |
| | | | 61 | 30 | 610 |

$r = 0.606$
$P < 0.001$

in maximum binding capacity correlated directly with plasma albumin concentration and Table 8 illustrates this finding, the association again being highly significant statistically ($P < 0.001$).

The affinity constant for phenytoin was analysed by $t$ test. No changes were observed with age, in keeping with the previous findings with warfarin.

The age effect on phenytoin clearance, therefore, could be related to an age effect on albumin concentration. When the products (C phenytoin) x plasma albumin for the young and old groups were compared, the difference of the means was not statistically significant ($P > 0.1$).

## Discussion

Changes in drug metabolism in the elderly have been previously observed in that the plasma half lives of phenylbutazone and antipyrine have been found to be lengthened in elderly people (O'Malley, Crooks, Duke & Stevenson, 1971). However, with both these drugs changes in the volume of distribution were noted between the young and elderly groups. The authors also noted changes between the sexes in that the half-life was more prolonged in males than in females.

Differences in absorption or protein binding were postulated as possible causes of this difference. Direct comparison of half-life between individuals in such circumstances may be misleading and estimations of clearance (which takes account of the volume of distribution) then provide a better method of comparison.

The calculation of clearance assumes a log-linear decay. At high blood concentrations evidence suggests that phenytoin does not always follow such a decay pattern (Arnold & Gerber, 1969). In our experiments the maximum plasma phenytoin concentration did not exceed 8 μg/ml in any patient and the decay curves observed were of a log-linear type. The calculation also assumes total utilisation of the dose of drug administered. If absorption is impaired erroneously high values will be obtained. In this study the increase in phenytoin clearance observed in the elderly group persists after intravenous administration, indicating that decreased absorption in the elderly does not account for this change.

Body distribution of highly bound drugs can be profoundly influenced by changes in protein binding capacity (Butler, 1971). Phenytoin itself is approximately 94% albumin bound (Lunde *et al.*, 1970) though this may not be the only binding protein *in vivo* (Lightfoot & Christian, 1966). However, albumin is probably the major binding protein and at the drug concentrations used in our experiments the Rosenthal plot indicated only one class of binding site.

Plasma albumin has been shown to fall in the elderly (Woodford-Williams, Alvarez, Webster, Landless & Dixon, 1964) and it has been previously postulated that a possible association exists between changes in plasma binding capacity and clearance and this has now been demonstrated.

An inverse correlation was also obtained between plasma albumin levels as measured by the H.A.B.A. dye method and clearance indicating that this is an age effect on plasma albumin. These clearance and binding changes persist in the group consisting solely of females. It would, therefore, seem that sex in itself is not of great importance apart from the fact that the albumin levels were lower in women particularly those over 65 years of age.

If due allowance is made for plasma albumin then no apparent age change in liver function is discernible indicating that the changes in albumin are the major factors contributing to this change in the elderly.

We gratefully acknowledge the help of Dr J. Ashley, Consultant Geriatrician, and Mrs L.T. White, technician in the Medical Unit.

This work was supported by the Nuffield Foundation as part of the Nottingham Old Age Project.

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*(Revised July 30, 1974)*