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# MODIFICATION OF PHENYTOIN CLEARANCE BY VALPROIC ACID IN NORMAL SUBJECTS

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1 The effect of valproic acid on the distribution and elimination kinetics of intravenously administered phenytoin has been investigated in eight normal volunteers.

2 In each of the subjects studied the volume of distribution of phenytoin increased significantly during treatment with sodium valproate (1200 mg daily for 7 days).

3 Phenytoin clearance was markedly increased in presence of valproic acid as compared to control values ($0.52 \pm 0.17$ *v* $0.38 \pm 0.11$ ml $min^{-1}$ $kg^{-1}$ respectively, $P < 0.02$).

4 It is suggested that the increase of the volume of distribution and of the serum clearance are secondary to displacement of phenytoin from plasma protein binding sites by valproic acid.

## Introduction

Valproic acid has been shown to displace phentoin from plasma protein-binding sites and to increase the unbound (free) fraction of phenytoin *in vitro* (Jordan, Shillingford & Steed, 1976; Monks, Boobis, Wadsworth & Richens, 1978; Patsalos & Lascelles, 1977). Since the pharmacological activity is proportional to the plasma concentration of free drug, it has been suggested that displacement from plasma protein-binding sites might enhance the clinical effects of phenytoin and precipitate phenytoin intoxication in epileptic patients receiving sodium valproate therapy (Patsalos & Lascelles, 1977). Extrapolation of *in vitro* data to the *in vivo* situation, however, has to be made cautiously because the displaced drug is available not only to produce pharmacological effects but also to be distrubuted in tissues and to be eliminated (Levy & Yacobi, 1974; Koch-Weser & Sellers, 1976; Shand, Mitchell & Oates, 1975). As phenytoin is one of those drugs which show flow-independent restrictive elimination (Blaschke, Meffin, Melmon & Rowland, 1975), i.e. only the unbound drug can be cleared, the increase in plasma concentration of unbound phenytoin after displacement by valproic acid would be expected to be transient and rapidly offset by a compensatory enhancement of the hepatic clearance of the drug (Gugler & Azarnoff, 1976; Dahlqvist, Borgå, Rane, Walsh & Sjöqvist, 1979). In order to examine the latter possibility the effect of valproic acid on the distribution and elimination kinetics of intravenously administered phenytoin has been investigated in normal volunteers.

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## Methods

Eight drug-free normal volunteers, (seven males, one female) aged between 21–39 years and informed of the nature of the study, were studied on two occasions separated by an interval of approximately 3 weeks. On one occasion phenytoin sodium 250 mg dissolved in the solvent of the commercial preparation (Epanutin®, Parke-Davis) were added to 200 ml 0.9% sodium chloride w/v and infused at a constant rate into an antecubital vein over a period of 30 min. Frequent blood samples were taken for up to 48 h following administration. On a second occasion each subject received sodium valproate (Depakine®, Sigma-Tau) 400 mg three times daily orally for 7 days. On day 6 phenytoin was administered intravenously according to the protocol described above. The order of treatments was randomized and the experimental conditions were maintained unchanged in respect to the time of dosing and to the time and composition of meals. No side effects were observed. Serum samples were kept frozen at −20°C until analysed for phenytoin and valproic acid concentration after the completion of the study. The concentration of phenytoin and valproic acid was determined in duplicate serum samples by gas-liquid chromatography according to Berlin, Agurell, Borgå, Lund & Sjöqvist (1972) and Schultz & Toseland (1977) respectively. The analyst

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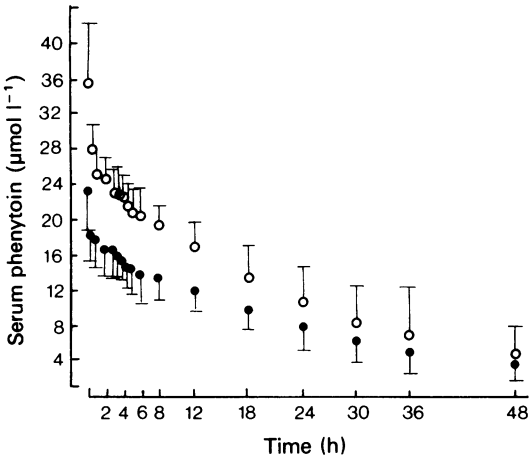


**Figure 1** Serum phenytoin concentration values (mean ± s.d.) following intravenous administration of phenytoin sodium (250 mg) in eight normal subjects. ○ control values, ● values determined during concurrent treatment with sodium valproate.

measuring the serum phenytoin concentration was kept unaware of the sequence of treatments.

Following intravenous administration serum phenytoin levels declined bi-exponentially in many of the subjects studied. Pharmacokinetic analysis according to a two-compartment open model, however, could not be satisfactorily performed because of slight irregularities during the early distributive phase of the serum concentration curves. Four to six hours after administration the decline of the log-concentration values with time appeared linear. The first-order rate constant of the terminal slope ($\beta$) and the serum half-life ($T_{\frac{1}{2}}$) were calculated from the latter values by linear regression. The area under the serum concentration curve (AUC) was determined by the trapezoidal rule and extrapolated to infinity. The total serum clearance (Cl) was calculated according to the model-independent relationship: Cl = Dose/AUC (Wilkinson & Shand, 1975). The volume of distribution ($V_d$) was calculated as Dose/(AUC × $\beta$) (Odar-Cederlöf & Borga, 1974). Statistical analysis was performed by means of the Student's *t*-test for paired data.

## Results

As illustrated in Figure 1 sodium valproate had a marked influence on the serum concentration profile of intravenousy administered phenytoin. At all sampling times serum phenytoin levels were considerably lower in presence of valproic acid as compared to control values. Calculated kinetic parameters are shown in Table 1. In all subjects the volume of distribution of phenytoin increased during treatment with sodium valproate. The terminal half-life remained substantially unchanged while the total body clearance increased by approximately 35% in presence of valproic acid. The increase of the serum clearance was observed in seven out of eight subjects and was statistically significant for the group as a whole.

Serum valproic acid levels (mean of two determinations of samples collected 2 and 6 h after the morning dose on day 6) ranged from 425 to 491 μmol/litre.

## Discussion

As phenytoin is almost exclusively eliminated by biotransformation (Hvidberg & Dam, 1976) the increase of the phenytoin clearance in presence of valproic acid is most likely to be due to enhancement of its metabolism. It is extremely unlikely that enzyme-induction is responsible for the observed interaction because sodium valproate is virtually devoid of enzyme-inducing properties in man (Perucca, Hedges, Makki, Hebdige, Wadsworth & Richens, 1979). The fact that the phenytoin half-life was not shortened during treatment with sodium valproate provides further evidence against induction of phenytoin metabolism. On the contrary, all available evidence supports our hypothesis that the increased phenytoin clearance is secondary to a plasma protein binding interaction. Several authors have previously shown that valproic acid at serum concentrations similar to those observed in the present study significantly displaces phenytoin from protein binding sites (Monks *et al.*, 1978; Patsalos & Lascelles, 1977) and increases by 30–100% its unbound fraction *in vitro* (Lecchini, Gatti, De Bernardi, Caravaggi, Frigo, Calzetti & Visintini, 1978; Monks, 1978) and *in vivo* (Dahlqvist *et al.*, 1979; Mattson, Cramer, Williamson & Novelly, 1978; Monks & Richens, 1979). Although the concentration of unbound drug could not be measured in the present study (due to accidental loss of the samples stored for this purpose), indirect evidence of displacement in our subjects is provided by the marked increase of the volume of distribution of phenytoin in presence of valproic acid. If the null hypothesis set up in our study that changes in distribution volume and clearance were solely caused by changes in protein binding is correct, then $AUC_{(free\ drug)}$ should remain constant in absence (1) and in presence (2) of valproic acid. Thus:

$$(1)\ AUC_1 \times = AUC_2 \times \alpha_2 = AUC_{(free\ drug)}$$

or by rearrangement:

$$(2)\ \frac{\alpha_2}{\alpha_1} = \frac{AUC_1}{AUC_2} = \frac{697}{516} = \frac{135}{100}$$

where $\alpha$ represents the free fraction of phenytoin in serum. Thus, according to the null hypothesis, the experimental results could be explained by an approximate 35% rise in the free fraction, which is well within the range of results reported in the literature.

According to pharmacokinetic theory displacement results in increase concentration of free drug in plasma. However, for a drug like phenytoin which has a relatively large volume of distribution and is subject to restrictive elimination such an effect should only be transient because the increased free drug concentration also increases the rate of elimination and of diffusion into tissues: this should result in reduced plasma concentration of total (free + bound) drug whereas the concentration of free drug should gradually revert to its initial value (Koch-Weser & Sellers, 1976; Shand *et al.*, 1975). Indeed an inverse relationship between degree of plasma protein binding and metabolic drug clearance in man has been demonstrated previously for warfarin (Yacobi, Udall & Levy, 1976) and phenytoin itself (Gugler & Azarnoff, 1976; Hayes, Langman & Short, 1975). The very marked increase of phenytoin clearance following single intravenous administration in elderly (Hayes *et al.*, 1975) and uraemic patients (Odar-Cederlöf & Borgå, 1974) with hypoalbuminaemia is likely to be due at least in part to reduced plasma protein binding of phenytoin in these conditions. Moreover, increased clearance of total phenytoin has been recently demonstrated in normal subjects following displacement of the drug from plasma proteins by salicylic acid (Fraser, Ludden, Evens & Sutherland, 1979). In this study, neither the AUC nor the total body clearance of free phenytoin were affected by the displacing agent. The results obtained in the present study suggest that a similar effect takes place in presence of valproic acid.

The interpretation that the enhancement of phenytoin clearance was related to a plasma protein binding interaction has received strong experimental support in a recent study by Mattson and coworkers (1978). In 21 epileptic patients the latter authors described a marked fall of the total serum phenytoin concentration a few days after starting valproic acid therapy: the effect was associated with a 60–100% increase of the unbound *fraction* of phenytoin in serum, whereas the *concentration* of free drug showed little or no change. In one patient who was more extensively investigated, initiation of valproic acid therapy resulted in an immediate and transient increase of the rate of excretion of the major phenytoin metabolite, 5,p-hydroxyphenyl-5-phenylhydantoin (*p*-HPPH), in urine. Conversely, a reduction of the urinary excretion of the metabolite was

**Table 1** Phentoin kinetic parameters following administration of a single intravenous dose of the sodium salt (250 mg)

| *Subject* | *Age (years)* | *Weight (kg)* | *β ($h^{-1}$)* | | *$T_{\frac{1}{2}}$ (h)* | | *AUC ($\mu mol\ l^{-1}\ h$)* | | *$V_d$ ($l\ kg^{-1}$)* | | *Cl ($ml\ min^{-1}\ kg^{-1}$)* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | a | b | a | b | a | b | a | b | a | b |
| 1 | 39 | 64 | 0.0281 | 0.0306 | 25 | 23 | 947 | 582 | 0.54 | 0.81 | 0.25 | 0.41 |
| 2 | 21 | 65 | 0.0617 | 0.0453 | 11 | 15 | 467 | 283 | 0.49 | 1.10 | 0.50 | 0.83 |
| 3 | 26 | 53 | 0.0241 | 0.0230 | 29 | 30 | 837 | 515 | 0.86 | 1.46 | 0.34 | 0.56 |
| 4 | 33 | 71 | 0.0221 | 0.0216 | 31 | 32 | 1025 | 816 | 0.57 | 0.73 | 0.21 | 0.26 |
| 5 | 25 | 67 | 0.0568 | 0.0469 | 12 | 15 | 516 | 395 | 0.47 | 0.74 | 0.44 | 0.58 |
| 6 | 22 | 62 | 0.0511 | 0.0476 | 14 | 15 | 514 | 495 | 0.56 | 0.63 | 0.48 | 0.50 |
| 7 | 37 | 57 | 0.0320 | 0.0446 | 22 | 16 | 744 | 430 | 0.68 | 0.84 | 0.36 | 0.62 |
| 8 | 28 | 65 | 0.0575 | 0.0393 | 12 | 18 | 524 | 608 | 0.47 | 0.59 | 0.45 | 0.39 |
| Mean | 29 | 63 | 0.0417 | 0.0374 | 19 | 20 | 697 | 516* | 0.56 | 0.86† | 0.38 | 0.52* |
| s.d. | 7 | 6 | 0.0166 | 0.0108 | 8 | 7 | 221 | 160 | 0.13 | 0.29 | 0.11 | 0.17 |

$\beta$ = rate constant of the terminal slope; $T_{\frac{1}{2}}$ = serum half-life; AUC = area under the curve; $V_d$ = volume of distribution; Cl = total body clearance; a = control values; b = during valproate treatment

* = $P < 0.02$; † = $P < 0.01$.

observed in the same subject when valproic acid was discontinued. These findings provide further evidence of increased phenytoin elimination at the onset of sodium valproate therapy. As expected the effect was only transient: once the concentration of free drug has returned to baseline levels the cause for increased elimination is removed and the amount of drug metabolized per unit of time also reverts to its initial value. Although the interaction could be complicated in presence of saturation kinetics, it is unlikely that this occurred at the low serum phenytoin concentration values observed in our study.

Apart from their interest in terms of a pharmacokinetic model the findings discussed above may have important clinical implications. The relative stability of the serum concentration of unbound phenytoin in presence of valproic acid, to which the increase in clearance observed in this study is likely to contribute, indicates that displacement is unlikely to produce any sustained change in the pharmacological activity of the former drug. The resulting marked increase of the volume of distribution of phenytoin in presence of valproic acid, however, must be kept in mind when interpreting serum phenytoin levels in clinical practice: despite the lower total drug concentration in serum, the amount of drug in tissues may not be reduced and dosage may not need to be increased.

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## References

BERLIN, A., AGURELL, S., BORGÅ, O., LUND, L. SJÖQVIST, F. (1972). Micromethod for the determination of diphenylhydrantoin in plasma and cerebrospinal fluid: a comparison between a gas-chromatographic and a spectrophotometric method. *Scand. J. clin. lab. Invest.*, **29**, 281–287.

BLASCHKE, T.F., MEFFIN, P.J., MELMON, K.L. & ROWLAND, M. (1975). Influence of acute viral hepatitis on phenytoin kinetics and protein binding. *Clin. Pharmac. Ther.*, **17**, 685–691.

DAHLQVIST, R., BORGÅ, O., RANE, A., WALSH, Z. & SJÖQVIST, F. (1979). Decreased plasma protein binding of phenytoin in patients on valproic acid. *Br. J. clin. Pharmac.*, (in press).

FRASER, D.G., LUDDEN, T., EVENS, R.P. & SUTHERLAND, E.W. III (1979). *In vivo* displacement of phenytoin from plasma proteins with salicylate. *Clin. Pharmac. Ther.*, **25**, 226.

GUGLER, R. & AZARNOFF, D.L. (1976). Drug protein binding and the nephrotic syndrome. *Clin. Pharmacokin.*, **1**, 161–188.

HAYES, M.J., LANGMAN, M.J.S. & SHORT, A.H. (1975). Changes in drug metabolism with increasing age. Phenytoin clearance and protein binding. *Br. J. clin. Pharmac.*, **2**, 73–79.

HVIDBERG, E.F. & DAM, M. (1976). Clinical pharmacokinetics of anticonvulsants. *Clin. Pharmacokin.*, **1**, 161–188.

JORDAN, B.J., SHILLINGFORD, J.S. & STEED, K.P. (1976). Preliminary observations on the protein-binding and enzyme-inducing properties of sodium valproate (Epilim). In: Legg, N.J. (ed.), *Clinical and Pharmacological Aspects of Sodium Valproate (Epilim) in the Treatment of Epilepsy* pp. 112–116 Tunbridge Wells: MCS Consultants.

KOCH-WESER, J. & SELLERS, E.M. (1976). Binding of drugs to serum albumin. *New Engl. J. Med.*, **294**, 526–531.

LECCHINI, S., GATTI, G., DE BERNARDI, M., CARAVAGGI, M., FRIGO, G., CALZETTI, S. & VISINTINI, D. (1978). Serum protein binding of diphenylhydantoin in man. 1—Interaction with sodium valproate. *Il Farmaco*—Ed. Pr., **33**, 80–82.

LEVY, G. & YACOBI, A. (1974). Effect of plasma protein binding on elimination of warfarin. *J. pharm. Sci.*, **63**, 805–806.

MATTSON, R.H., CRAMER, J.A., WILLIAMSON, P.D. & NOVELLY R.A. (1978). Valproic acid in epilepsy: clinical and pharmacological effects. *Ann. Neurol.*, **3**, 20–25.

MONKS, A. (1978). *Binding of phenytoin and valproic acid by human serum proteins*. Ph.D. Thesis, University of London.

MONKS, A., BOOBIS, S., WADSWORTH, J. & RICHENS, A. (1978). Plasma protein binding interaction between phenytoin and valproic acid *in vitro*. *Br. J. clin. Pharmac.*, **6**, 487–492.

MONKS, A. & RICHENS, A. (1979). Effect of a single dose of sodium valproate on serum phenytoin concentration and protein binding in epileptic patients. *Clin. Pharmac. Ther.* (in press).

ODAR-CEDERLOF, I. & BORGÅ, O. (1974). Kinetics of disphenylhydantoin in uraemic patients: consequences of decreased plasma protein binding. *Eur. J. clin. Pharmac.*, **7**, 31–37.

PATSALOS, P.N. & LASCELLES, P.T. (1977). Effect of sodium valproate on plasma protein binding of diphenylhydantoin. *J. Neurol. Neuros. Psych.*, **40**, 570–574.

PERUCCA, B., HEDGES, A.M., MAKKI, K., HEBDIGE, S., WADSWORTH, J. & RICHENS, A. (1979). The comparative enzyme-inducing properties of antiepileptic drugs. *Br. J. clin. Pharmac.*, **7**, 414–415P.

SHAND, D.G., MITCHELL, J.R. & OATES, J.A. (1975). Pharmacokinetic drug interactions. In: Gillette, J.R., Mitchell, J.R. & Randall, P.S. (eds), *Handbook of Experimental Pharmacology*, Vol. 28 (3), pp. 272–314, Berlin, Heidelberg, New York: Springer-Verlag.

SCHULTZ, F-U. & TOSELAND, P.A. (1977). Determination of the anti-convulsant drug sodium di-n-propylacetate in human plasma by gas-chromatography. *Ann. clin. Biochem.*, **14**, 240–242.

WILKINSON, G.R. & SHAND, D.G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmac. Ther.*, **18**, 377–399.

YACOBI, A., UDALL, J.A. & LEVY, G. (1976). Serum protein binding as determinant of warfarin body clearance and anticoagulant effect. *Clin. Pharmac. Ther.*, **19**, 552-558.

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