

Inhibition of phenobarbitone *N*-glucosidation by valproate

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- 1 Plasma phenobarbitone concentrations and daily urinary excretion of phenobarbitone and its metabolites *p*-hydroxyphenobarbitone (conjugated and unconjugated), and [S]-phenobarbitone-*N*-glucoside were measured under steady-state conditions in two groups of epileptic patients, (i) taking phenobarbitone with or without other drugs, but not valproate ($n = 12$), and (ii) taking phenobarbitone with other drugs including valproate ($n = 8$).
- 2 Mean steady-state plasma phenobarbitone concentrations were 5.9 mg l⁻¹ higher, relative to drug dose, in the patients taking valproate than in those not taking valproate.
- 3 Urinary excretion of [S]-phenobarbitone-*N*-glucoside was significantly lower in the group taking valproate ($1.9 \pm \text{s.d. } 2.0\%$ of phenobarbitone dose vs $16.2 \pm \text{s.d. } 9.9\%$). Urinary excretion of phenobarbitone ($23.7 \pm \text{s.d. } 9.8\%$ vs $48.2 \pm \text{s.d. } 13.6\%$) and unconjugated *p*-hydroxyphenobarbitone ($5.7 \pm \text{s.d. } 3.9\%$ vs $16.0 \pm \text{s.d. } 9.1\%$) was higher in those taking valproate, while conjugated *p*-hydroxyphenobarbitone excretion was similar in both groups ($8.3 \pm \text{s.d. } 4.9\%$ vs $6.5 \pm \text{s.d. } 2.9\%$).
- 4 Valproate appeared to inhibit both the direct *N*-glucosidation of phenobarbitone and the *O*-glucuronidation of *p*-hydroxyphenobarbitone.

Keywords valproate phenobarbitone interaction inhibition glucosidation

Introduction

Phenobarbitone and valproic acid are sometimes prescribed in combination for the control of seizure disorders [1]. There is a well-recognized pharmacokinetic interaction between these drugs, which has been documented in two ways. Firstly, addition of valproate to the therapy of patients at steady state for phenobarbitone typically causes a rise in the steady-state plasma phenobarbitone concentration [2–10]. Secondly, in population studies, patients taking phenobarbitone plus valproate tend to have higher plasma phenobarbitone concentrations relative to dose than those taking phenobarbitone without concurrent valproate [11]. If this interaction results in sedation, the valproate dose may be reduced or ceased, when the more appropriate course might be to reduce the phenobarbitone dose, guided by knowledge of its plasma concentrations before and after the introduction of valproate.

While this interaction has been widely noted, its underlying mechanism has not been fully elucidated. It has been established that the metabolic clearance of

phenobarbitone is lower in the presence of valproate [7, 9], but it is not clear which metabolic pathway(s) are affected.

Phenobarbitone is eliminated partly by hepatic metabolism and partly by renal excretion. The major known pathways of metabolism are *p*-hydroxylation in the 5-phenyl substituent, yielding a phenolic metabolite (*p*-hydroxyphenobarbitone) which is excreted in urine predominantly as its *O*-glucuronide conjugate [12], and conjugation with glucose giving a *N*-glucoside which is present mainly as the [S]-diastereomer [13, 14].

Kapetanovic & Kupferberg [15] showed that valproate inhibited the *p*-hydroxylation of phenobarbitone in rat hepatic microsomal preparations, a finding which was recently confirmed by Anderson & Levy [16]. However, the inhibition constant determined by the former authors in rat studies seemed too high to account for the inhibitory effects seen clinically in humans, and the latter authors concluded that inhibition of the formation of *p*-hydroxyphenobarbitone

could not account fully for the increased plasma concentrations of phenobarbitone that occur when valproate is added to phenobarbitone therapy.

We have undertaken studies of the elimination of phenobarbitone in chronically treated epileptic patients which go some way towards explaining the mechanism of the interaction between valproate and phenobarbitone.

Methods

Subjects studied and study protocol

The study was carried out in two groups of epileptic patients whose details are shown in Table 1. The first group comprised 12 persons (5 male, 7 female; mean age $48.3 \pm$ s.d. 16.5 years; age range 28 to 83 years; mean weight $71.0 \pm$ s.d. 11.5 kg), three of whom received phenobarbitone as anticonvulsant monotherapy while the remaining nine took the drug with anticonvulsants other than valproate. The second group comprised eight subjects (four male, four female; mean age $42.8 \pm$ s.d. 15.3 years; age range 20 to 74 years; mean weight $74.9 \pm$ s.d. 19.0 kg), who were treated with both phenobarbitone and valproate (three with other anticonvulsants also). The mean daily phenobarbitone dose in the first group was $124 \pm$ s.d. 34 mg; range 90–200 mg; in the second group the mean daily phenobarbitone dose was $118 \pm$ s.d. 57 mg; range 60–240 mg, and the mean daily val-

proate dose was $2275 \pm$ s.d. 1284 mg; range 500–4000 mg.

All subjects were studied only after they had taken a constant dosage for a sufficient period for steady-state conditions to apply. Venous blood was collected during the urine collection interval for measurement of plasma phenobarbitone concentration. All urine was collected over periods of 3 consecutive days by five subjects (D, F, G, H, I), 2 days by five subjects (A, B, C, E, L), and one day only by two subjects (F, K) in the first groups, and over 2 consecutive days by six subjects (M, O, Q, R, S, T) and one day only by two subjects (N, P) in the second group. The urine was collected into bottles containing citric acid (10 g), since phenobarbitone-*N*-glucoside is known to be unstable at pH > 5 [17]).

Analysis of plasma and urine samples

Plasma phenobarbitone concentrations were determined as part of the patients' routine management. The assays were performed by the Pathology Department, Royal Brisbane Hospital, using a validated TDX method. The urinary concentrations of phenobarbitone, *p*-hydroxyphenobarbitone and [S]-phenobarbitone-*N*-glucoside were measured by h.p.l.c. Unconjugated *p*-hydroxyphenobarbitone was measured by assay of urine directly, and total *p*-hydroxyphenobarbitone after hydrolysis at 37°C for 17 h with 1000 Fishman units of β -glucuronidase (from *Helix pomatia*, Type H-2, in phosphate buffer, 0.1 M, pH 5), with the conjugated metabolite then

Table 1 Personal details and drug therapy of subjects studied

Subject	Age (years)	Sex	Weight (kg)	PB	Daily dose (mg)			Other drugs
					VPA	PHT	CBZ	
<i>Group I – phenobarbitone, no valproate</i>								
1	39	F	57	90				TZP, PCM, PET, MCP, COD
2	37	M	79	120		400		
3	28	M	80	120		300		CZP
4	28	M	80	200		300		DZP
5	56	F	90	120		400		DZP, CS
6	43	F	58	90			800	DZP
7	45	F	60	90				CZP, TZP, PRM, MHD, RND
8	62	M	82	120		600		CMD
9	47	F	57	120			1000	FA
10	69	F	64	120		300		FA, CAC, NBC, CPT
11	83	M	75	180				
12	43	F	70	120				TMP
<i>Group II – phenobarbitone plus valproate</i>								
13	36	M	80	240	3500		600	DZP, CZP, PRD, VGT
14	74	M	81	90	500			NFP, CMD, PRD, WAR
15	36	F	77	120	2500			AMX, MDZ, TFD
16	49	F	55	90	1200	300		DZP
17	20	F	48	60	1500			
18	45	M	95	75	4000	600		
19	39	F	61	150	3500			
20	43	M	102	120	1500			VGT, LTG

Key: AMX, amoxicillin; CAC, Ca carbonate; CBZ, carbamazepine; CMD, cimetidine; COD, codeine; CPT, cephalothin; CS, Ca supplements; CZP, clonazepam; DZP, diazepam; FA, folic acid; LTG, lamotrigine; MCP, metoclopramide; MDZ, methdilazine; MHD, methadone; NBC, Na bicarbonate; NFP, nifedipine; PB, phenobarbitone; PCM, paracetamol; PET, pethidine; PHT, phenytoin; PRD, prednisone; PRM, conjugated equine oestrogens; RND, ranitidine; TFD, terfenadine; TMP, trimethoprim; TZP, temazepam; VGT, vigabatrin; VPA, sodium valproate; WAR, warfarin.

being determined by difference. The analytes were extracted from acidified urine (or urine hydrolysate) with ethyl acetate, the organic layer transferred to a clean tube and evaporated to dryness, and the residue reconstituted in an aliquot of a solution prepared by mixing orthophosphoric acid (1.0 M; 1.0 ml), acetonitrile (12 ml) and distilled water (87 ml). An aliquot of this reconstituted solution was injected into the h.p.l.c. Full details of this procedure are reported elsewhere [18].

The h.p.l.c. system comprised a model 510 pump, a RCM-100 radial compression module containing a 4 µm Novapak C₁₈ cartridge preceded by a guard column containing Bondapak C₁₈ Corasil, a model 481 LC Spectrophotometer (all from Waters Associates, Milford, MA), a model K 65B automated sample injector (ETP Kortec Pty Ltd, Sydney, Australia) and a model C-R3A Chromatopac integrator with FDD-1A floppy disk drive and CRT screen (Shimadzu Corp., Kyoto, Japan). The mobile phase was methanol: phosphate buffer (0.025 M; pH 5) (23:77). Flow was at 1.4 ml min⁻¹ in the recycling mode, with the phase being renewed every 3 days or as required. Column eluent was monitored at 220 nm. Under these conditions the retention times were approximately 12, 28, 34, 39 and 66 min for *p*-hydroxyphenobarbitone, [S]-phenobarbitone-*N*-glucoside, [R]-phenobarbitone-*N*-glucoside, phenobarbitone and 5-allyl-5-phenylbarbituric acid (internal standard), respectively. The limit of quantification was 0.2 µg ml⁻¹ for phenobarbitone and *p*-hydroxyphenobarbitone, and 1 µg ml⁻¹ for [S]-phenobarbitone-*N*-glucoside. The intra-assay variability was < 7% for all analytes at low concentrations (2 µg ml⁻¹) and < 4% at higher concentrations (10 and 40 µg ml⁻¹). The corresponding values for inter-assay variability were < 9% and < 5%.

The statistical significances of differences between means were determined with the aid of the Confidence Interval Analysis microcomputer program of Gardner & Altman [19].

Results

Linear regressions were calculated for plasma phenobarbitone concentration on phenobarbitone dose for the group of subjects not receiving, and for the group concurrently receiving valproate (Figure 1). The two regressions did not differ in slope (0.066 and 0.077, respectively; difference = 0.0107; 95% C.I. = -0.131 to 0.152), but differed in elevation (difference = 5.87 mg l⁻¹; 95% C.I. = 0.160 to 11.6 mg l⁻¹). Thus plasma phenobarbitone concentrations were statistically significantly higher in the subjects receiving phenobarbitone plus valproate than in those receiving phenobarbitone without valproate, over the range of concentrations studied.

Steady-state 24 h urinary excretion of phenobarbitone and its metabolites (expressed as percentages of the molar dose of phenobarbitone taken) in individual members of the two groups of subjects are shown in Table 2. The mean percentage of the daily dose of

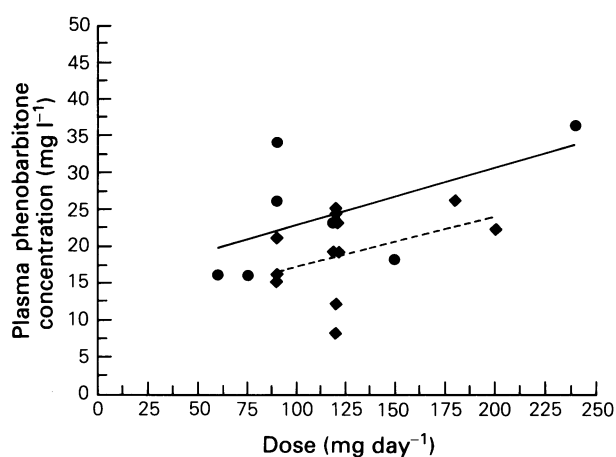


Figure 1 Regressions of steady-state plasma phenobarbitone concentration on daily phenobarbitone dose for patients taking phenobarbitone with (solid circles, continuous line) and without valproate (solid diamonds, broken line). The regression equations are, respectively, $y = 0.0767 + 0.0453x$ and $y = 0.0660 + 0.475x$. No plasma concentration value was available for Subject 20.

phenobarbitone excreted unchanged in the group taking valproate with phenobarbitone was approximately twice that in the group taking phenobarbitone without concurrent valproate (48.2% v 23.7%), the difference (24.6%) having a 99% C.I. of 9.58% to 39.6%. The mean percentage excreted as *p*-hydroxyphenobarbitone glucuronide was similar in those taking phenobarbitone with (6.50%) and without (8.3%) valproate, the difference of 1.77% having a 95% C.I. of -2.29% to 5.83%. However, the mean percentage of the dose excreted as non-conjugated *p*-hydroxyphenobarbitone was substantially higher in those taking phenobarbitone with valproate (16.0%) than in those taking phenobarbitone but not taking valproate (5.7%; difference = 10.3%; 99% C.I. = 1.79% to 18.7%). Offsetting this greater proportional excretion of unchanged phenobarbitone and total *p*-hydroxyphenobarbitone in those taking phenobarbitone with valproate, the mean excretion of [S]-phenobarbitone-*N*-glucoside in this group (1.9%) was nearly an order of magnitude less than that for subjects taking phenobarbitone but not taking valproate (16.3%; difference = 14.3%; 99% C.I. = 4.01 to 24.6%). No measurable [R]-phenobarbitone-*N*-glucoside was present in the urines of those taking valproate. The total percentage of the phenobarbitone dose accounted for as urinary excretion products was higher in those co-medicated with valproate (72.6 ± s.d. 11.1%) than in those taking phenobarbitone but not valproate (53.8 ± s.d. 19.8%; difference = 18.8%; 95% C.I. = 2.50 to 31.5%).

There were no statistically significant correlations between the percentage of the phenobarbitone dose excreted as [S]-phenobarbitone-*N*-glucoside, or between the percentage of the dose excreted as unconjugated *p*-hydroxyphenobarbitone, and the valproate dose.

Table 2 Steady state 24 h urinary excretion of phenobarbitone and its metabolites, expressed as percentage of the molar dose of phenobarbitone taken

Subject	phenobarbitone	% of dose excreted as		[S]-phenobarbitone-N-glucoside
		<i>p</i> -hydroxyphenobarbitone conjugated	non-conjugated	
<i>Group I – phenobarbitone, no valproate</i>				
1	27	13.3	13.7	16
2	30	5.2	3.8	24
3	37	4.9	3.1	34
4	16	8.9	4.1	9
5	32	5.3	2.7	11
6	12	10.8	7.2	24
7	32	20.9	9.1	25
8	25	7.9	2.1	24
9	34	4.4	7.6	6
10	8	5.6	1.4	1
11	14	3.9	3.1	12
12	17	8.1	10.9	8
Mean ± s.d.	23.7 ± 9.8	8.3 ± 4.9	5.7 ± 3.9	16.2 ± 9.9
<i>Group II – phenobarbitone plus valproate</i>				
13	47	4	20	2
14	46	4	14	4
15	49	12	37	0
16	72	6	9	0
17	51	8	25	5
18	30	5	37	3
19	59	4	19	0
20	32	9	19	1
Mean ± s.d.	48.2 ± 13.6	6.5 ± 2.9	16.0 ± 9.1	1.9 ± 2.0

Discussion

There have been previous attempts to determine the mechanism of the well documented phenobarbitone-valproate interaction in humans. Patel *et al.* [7] showed that the interaction involved a decrease in the plasma clearance but not in the renal clearance of phenobarbitone, indicating that the drug's metabolic clearance was reduced. A greater proportion of the phenobarbitone dose was excreted unchanged in urine, but it was not clear which metabolic pathway was inhibited. Kapetanovic *et al.* [9] obtained essentially similar pharmacokinetic and urinary phenobarbitone excretion data in three subjects, and in addition showed there was increased urinary excretion of unconjugated but not of total *p*-hydroxyphenobarbitone. Again, the data available did not permit identification of the phenobarbitone metabolic pathway which was inhibited by valproate. In rat liver microsomes, Kapetanovic & Kupferberg [15] showed that valproate inhibited the *p*-hydroxylation of phenobarbitone. Later Anderson & Levy [16] studied the interaction in rats. In these animals, the plasma clearance of phenobarbitone was decreased and the formation clearance of *p*-hydroxyphenobarbitone was decreased. The authors concluded that the main effect of valproate on phenobarbitone elimination in the rat must be on a metabolic pathway other than the elimination mechanisms studied *viz.* renal excretion of the unchanged drug and formation of phenolic deriva-

tives. The remaining known pathway of phenobarbitone elimination in humans is *N*-glucoside formation [20, 21], but this is not a major metabolic pathway in the rat [21]. Because of this Anderson & Levy [16] regarded altered phenobarbitone-*N*-glucosidation as unlikely to explain the phenobarbitone-valproate interaction.

There is reason to believe that urinary phenobarbitone-*N*-glucoside excretion may not reflect the true extent of phenobarbitone-*N*-glucoside formation, at least in humans [18]. The *N*-glucoside is chemically unstable at pH values above 5 [17], forming ring opened derivatives which would probably not be detected in the usual phenobarbitone-*N*-glucoside assays. Appreciable decomposition of phenobarbitone-*N*-glucoside at tissue, plasma and probably urine pH might be anticipated before this metabolite was excreted from the body. Hence, urinary [S]-phenobarbitone-*N*-glucoside measurements might underestimate significantly the capacity of glucosidation as a phenobarbitone elimination pathway. Because of this possibility, and because *N*-glucosidation had not been studied in humans in relation to the valproate-phenobarbitone interaction, it seemed worth reinvestigating the basis of this interaction in metabolic balance studies in chronically treated patients, assaying the parent drug and also its phenolic and *N*-glucoside metabolites.

The patients in the present study who were co-medicated with valproate appeared to have experienced the typical valproate-phenobarbitone interaction in that their plasma phenobarbitone concentrations relative to drug dose averaged some 33% higher than those in patients taking phenobarbitone but not valproate. As judged by the urinary metabolite excretion data, the patients exhibiting the interaction seemed to have almost completely lost their capacity to form the *N*-glucoside of phenobarbitone. They excreted increased proportions of their phenobarbitone doses as unchanged drug and as its main phenolic metabolite. While there was apparent diversion of phenobarbitone metabolism from *N*-glucosidation to phenolic derivative formation in those taking valproate, the capacity for glucuronidation of this Phase I metabolite was not increased in parallel with its increased formation. Thus, it appeared that valproate inhibited both the *N*-glucosidation of phenobarbitone and the *O*-glucuronidation of *p*-hydroxyphenobarbitone, the former reaction more than the latter. Although the majority of subjects taking valproate with phenobarbitone also received other drugs which might have influenced the findings, the two subjects (17 and 19) receiving only phenobarbitone and valproate also excreted very little phenobarbitone-*N*-glucoside.

In persons taking phenobarbitone without valproate a mean of 54% of the daily phenobarbitone dose could be accounted for in urine as parent substance and measured products of phenol and *N*-glucoside formation. In contrast, when valproate was taken together with phenobarbitone a mean of 73% of the dose could be accounted for as these same substances. The studies were done in circumstances which should have ensured steady-state conditions, and the mean daily phenobarbitone doses in the two patient groups studied were similar (124 and 118 mg). Particularly if, as mentioned above, urine phenobarbitone-*N*-glucoside excretion ($16.2 \pm \text{s.d. } 9.9\%$ of the dose) significantly underestimates the formation of this metabolite, inhibition of glucoside formation could have made enough phenobarbitone avail-

able for excretion unchanged or after phenolic derivative formation to account for the apparent discrepancy.

We have traced no previous reports of valproate inhibiting drug glucosidation. In animals, valproate is known to inhibit the glucuronidation of certain substrates, including *p*-hydroxyphenobarbitone [16, 22] and, in humans, of lamotrigine [23]. This inhibition appears likely to be due to depletion of hepatic UDP-glucuronic acid by valproate [24]. Such inhibition could explain why the increased formation of *p*-hydroxyphenobarbitone resulting from valproate-mediated inhibition of phenobarbitone-*N*-glucosidation was not associated with increased production of *p*-hydroxyphenobarbitone-*O*-glucuronide in patients taking valproate. Possibly valproate depletes not only the hepatic content of UDP-glucuronic acid, but also the content of its metabolic precursor UDP-glucose (salicylamide depletes the mouse liver of both [24]). If so, this could explain both the decreased *N*-glucosidation and *O*-glucuronidation found in the present investigation of the phenobarbitone-valproate interaction. There is, however, in the literature one finding which is inconsistent with this explanation. If valproate caused a depletion of UDP-glucuronic acid, it would be expected that all glucuronidations would be inhibited, regardless of which glucuronosyl transferase isozyme was involved in their catalysis. However, Kapetanovic *et al.* [9] showed that the glucuronidation of paracetamol was not inhibited by valproate in humans.

While the present study has helped explain the mechanism of the metabolic interaction between valproate and phenobarbitone, further work is indicated to achieve a more complete understanding of the effects of valproate on the conjugation reactions of other drugs.

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