VALPROIC ACID AND DIAZEPAM INTERACTION *IN VIVO*

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- 1 The effect of oral administration of sodium valproate (1500 mg daily) on the distribution and elimination kinetics of intravenously administered diazepam in six healthy volunteers has been studied.
- 2 During valproate administration the unbound fraction of diazepam in serum increased approximately two fold. This was accompanied by a significant increase in apparent volume of distribution and plasma clearance of diazepam.
- 3 There was a positive correlation between the change in free fraction and the increase in both apparent volume of distribution and plasma clearance of the drug.
- 4 The concentration of unbound diazepam in serum (calculated from the percent free diazepam and total serum concentration) was significantly higher during valproate administration. Both the intrinsic clearance and volume of distribution of unbound drug were significantly reduced.
- 5 Mean serum *N*-desmethyldiazepam levels were significantly lower during valproate coadministration.
- 6 These results suggest that valproic acid displaces diazepam from plasma protein binding sites and inhibits its metabolism.

Introduction

The pharmacokinetics of diazepam have been extensively investigated. Diazepam is approximately 98% bound to plasma proteins (van der Kleijn *et al.*, 1979; Thiessen *et al.*, 1976; Klotz *et al.*, 1976). Sjöholm *et al.* (1979) have shown that several benzodiazepines, halogenated derivatives of penicillin, antirheumatic drugs and tryptophan displace diazepam significantly from human serum albumin immobilised in micro-particles of polyacrylamide (Sjöholm *et al.*, 1979). Free fatty acids have also been shown to displace diazepam *in vitro* (Tsutsumi *et al.*, 1975; Sjödin, 1977) and *in vivo* (Colburn & Gibaldi 1978; Wong & Sellers 1979; Sellers *et al.*, 1980). Systemic administration of heparin, which increases non-esterified fatty acid concentration, also increases the unbound fraction of diazepam in subjects (Routledge *et al.*, 1980).

Valproic acid is a drug widely used in the treatment of both major and minor seizures (Gugler & Von Unruh, 1980). Chemically it is a two chain fatty acid which has similar properties to endogenous free fatty acids, including the ability to bind to fatty acid binding sites on albumin. Valproic acid has been shown to displace diazepam from plasma protein binding sites *in vitro* (Sjöholm *et al.*, 1979; Dhillon & Richens, 1981). Diazepam is a drug which shows

flow-independent restrictive elimination (Klotz *et al.*, 1976) i.e. the total metabolic clearance is directly proportional to the free fraction of the drug in blood (Shand *et al.*, 1975).

In order to examine the plasma protein binding interaction between diazepam and valproic acid *in vivo*, we have studied the effect of concurrent administration of sodium valproate on the distribution and elimination kinetics of intravenously administered diazepam in six normal volunteers.

Methods

Six healthy male volunteers, aged between 21–35 years participated in the trial. None was receiving regular drug treatment. Each subject was instructed to take a light breakfast of toast and fruit juice on the morning of each test day. Meals were not standardised over the two periods of the trial, but the subjects were asked to avoid a high fat intake. They were also asked to refrain from heavy physical exercise during the test period.

Each subject received on two different occasions, separated by an interval of at least 2 weeks, a single

intravenous injection of diazepam 10 mg (Valium, Roche). On one occasion no other drug was administered, whereas on the second occasion sodium valproate (Epilim, Labaz) was administered orally, 500 mg three times daily for 5 days. The first dose of sodium valproate was given approximately 20 h before the intravenous injection of diazepam.

Blood samples (5 ml–20 ml) were collected frequently on day 1 from the arm not used for intravenous injection using an indwelling butterfly needle. A heparin (Pularin, Duncan Flockhart) saline solution (2 units/ml) was used at each sampling time to flush the butterfly needle; no more than 5 ml was used on each occasion. This concentration of heparin is well below that known to influence diazepam binding to serum proteins (Desmond *et al.*, 1980; Naranjo *et al.*, 1980). Blood samples were taken before the injection and 2, 5, 10, 20, 30, 60, 90, 120 min and 4, 8, 12, 24, 28, 32, 48, 56, 72, 80, and 96 h after drug administration. The samples were left to clot for 1–2 h, centrifuged and the serum samples frozen in aliquots of 2–10 ml for drug and binding assays. All samples were stored at -20°C and assayed within 3 months.

Analytical methods

Serum diazepam and *N*-desmethyldiazepam concentrations were determined by gas liquid chromatography using a Perkin Elmer F33 gas chromatograph with electron capture detector. A glass column 3 m $\times$ 3 mm i.d. was packed with Kovats 3% C87 on Chromosorb W 100–120 mesh and maintained in the oven at 255°C . The temperature of the injector port and detector was 300°C . Carrier gas (N_2) flow was 120 ml min^{-1} . The extraction procedure followed was as described by Rutherford (1977). Within assay coefficients of variation between batches were found to be 4.4% and 2.2% for serum samples containing 25 ng ml^{-1} and 500 ng ml^{-1} respectively.

Valproic acid concentration in serum was determined by gas liquid chromatography according to the method of Schultz & Toseland (1977), in the 20 ml samples collected over the 80 h period following diazepam administration.

Diazepam binding to serum proteins was assessed by direct ultrafiltration according to Lunde *et al.* (1970). Serum aliquots (5 ml) were spiked with [^{14}C]-diazepam (206 $\mu\text{Ci}/\text{mg}$, purity $> 99\%$) at a concentration of 147 ng ml^{-1} and incubated for 45 min at room temperature. Following incubation duplicate aliquots (2 ml) were subjected to ultrafiltration through Visking tubing (Scientific Supplies) by centrifuging at 1000 g for 7 min and then 35 min. The 7 min ultrafiltrate was discarded and triplicate 70 μl aliquots from the 35 min ultrafiltrate were added to scintillation vials containing 10 ml of Packard Instagel Scintillant. The vials were counted for 10 min on a

Packard 2425 scintillation spectrometer. The unbound fraction was calculated as the ratio of disintegrations per minute (d/min) in the ultrafiltrate to the d/min in the serum before ultrafiltration.

Adsorption of diazepam to the Visking tubing was examined at a total serum diazepam concentration of 300 ng ml^{-1} . Visking bags containing 2 ml serum spiked with [^{14}C]-diazepam were suspended in the centrifuge tubes and were left for 2 h. Three 70 μl aliquots of the serum before and after storage were added to scintillation vials and counted for 10 min as described above. The mean adsorption was found to be $1.1 \pm 0.054\%$ after a 2 h storage period.

Kinetic analysis

Serum diazepam concentrations following intravenous administration declined biexponentially and could be adequately described according to a two compartment open model in all subjects. According to this model the serum concentrations at time (*t*) after a single intravenous dose is given by the equation

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where α and β are the rate constants of the initial and terminal phase respectively and *A* and *B* are constants. The β slope was obtained by linear regression from the terminal part of the serum concentration time curve and extrapolated back to zero time.

Half-lives ($T_{1/2}\alpha$, $T_{1/2}\beta$) were calculated according to this model. The area under the serum concentration time curve (AUC) was estimated using the trapezoidal rule and extrapolated to infinity. Total body clearance (Cl) was calculated by using the model independent relationship.

$$\text{Cl} = \frac{\text{Dose}}{\text{AUC}_0^\infty} \quad (\text{Gibaldi } et al., 1969).$$

Volume of distribution (Vd) of total (*t*) and free (*f*) drug was calculated as

$$\text{Vd}_t = \frac{\text{Dose}}{\text{AUC}_0^\infty \alpha \beta} \quad \text{Vd}_f = \frac{\text{Dose}}{\text{AUC}_0^\infty \beta'_{\text{free}}}$$

(β' was calculated from the unbound serum diazepam concentration time profile). In all subjects the extrapolated areas to infinity represented $13.2 \pm 8\%$ of the total area.

Statistical analysis was performed using the Student's *t*-test for paired observations.

Results

The mean total serum diazepam concentration-time profile for all subjects following intravenous administration is shown in Figure 1. After an initial distri-

bution phase which was virtually complete after 6–12 h, the decline of log serum diazepam concentration with time profile appeared linear. In the presence of valproic acid, the serum diazepam concentration was lower at each sampling point, in all subjects up to 96 h. There was no apparent influence on the slope of the elimination phase, which remained virtually parallel to that on the control days.

Figure 1 Mean $\pm$ s.d. serum diazepam concentrations in six subjects following intravenous administration of diazepam 10 mg in the absence of ($\blacktriangle$), and during concurrent administration of sodium valproate ($\bullet$).

Figure 2 illustrates the mean serum *N*-desmethyldiazepam concentration-time profile in all subjects. Serum *N*-desmethyldiazepam levels were significantly lower at each sampling point in all subjects during concurrent sodium valproate administration ($P < 0.05$).

Figure 2 Mean $\pm$ s.d. serum *N*-desmethyldiazepam concentrations in six subjects following intravenous diazepam 10 mg in the absence (Δ) and presence of valproate ($\circ$).

Diazepam serum protein binding was determined in samples taken at 10 min, 30 min, 4 and 8 h on day 1 and then at 24, 32, 48, 56, 72 and 80 h following intravenous administration of diazepam. The free diazepam fraction in each subject during the two test periods is shown in Table 1. In all subjects the binding of diazepam to serum proteins was markedly and consistently reduced at all sampling times. The mean

Table 1 Free diazepam fraction in the absence and presence of valproic acid (VA) following intravenous diazepam (10 mg) in six subjects. Mean $\pm$ s.d. results, $n = 10$.

Subject	Diazepam only (% free)	Diazepam + VA (% free)	Serum VA concentration ($\mu\text{g ml}^{-1}$)
1	1.73 ± 0.19	2.38 ± 0.28	66 ± 5
2	1.68 ± 0.16	3.10 ± 0.37	70 ± 11
3	1.68 ± 0.13	3.79 ± 0.55	77 ± 8
4	$\dagger 1.69 \pm 0.13$	$\dagger 3.02 \pm 0.51$	$\dagger 72 \pm 3$
5	1.81 ± 0.16	3.13 ± 0.44	73 ± 5
6	1.90 ± 0.18	4.13 ± 0.66	64 ± 8
Mean $\pm$ s.d.	1.75 ± 0.17	$* 3.36 \pm 0.75$	69 ± 15

$\dagger$ Mean of 9 samples; * $P < 0.01$

free diazepam fraction was $1.75 \pm 0.17\%$ and during concurrent sodium valproate administration the free fraction was significantly larger than that observed on the control days (Table 1). Mean % change in free fraction was $93 \pm 34\%$. The mean $\pm$ s.d. serum valproic acid concentration in all subjects over the 80 h period was $69 \pm 15 \mu\text{g ml}^{-1}$ (Table 1). There was a poor intra- and inter-subject correlation between % change in diazepam free fraction and serum valproic acid concentration. Mean unbound serum diazepam levels in the absence and presence of valproic acid are shown in Figure 3. Values have been calculated from

Figure 3 Mean $\pm$ s.d. calculated unbound serum diazepam concentrations in six subjects after intravenous diazepam 10 mg in the absence (▲) and presence (●) of valproate.

total serum diazepam concentrations and the unbound fraction of diazepam measured in the same samples. In all subjects, in the presence of valproic acid, diazepam serum concentrations were consistently higher than after diazepam alone. The area under the serum unbound diazepam concentration curve was significantly higher during concurrent sodium valproate administration ($P < 0.01$) (Table 2). Kinetic parameters calculated from the total serum diazepam concentration in each of the subjects are presented in Table 2.

Concurrent sodium valproate administration reduced the total serum diazepam levels in all subjects. Area under the serum concentration time curve $\text{AUC}_{0-\infty}$ was significantly lower in the presence of

valproic acid than in its absence. Serum diazepam half-life ($T_{1/2\beta}$) was not affected by sodium valproate co-administration. Total serum clearance and apparent volume of distribution were significantly higher in the presence of valproic acid. Figures 4 and 5 illustrate a positive rank order correlation between the % change in free fraction and both the increase in total plasma clearance ($r = 0.94$, $P < 0.02$) and the increase in apparent volume of distribution ($r = 0.94$, $P < 0.02$). However, in each subject studied the percentage increase in plasma clearance after sodium valproate administration was lower than the corresponding change in the unbound fraction of the drug.

Figure 4 Relationship between change in free diazepam fraction and percent increment in apparent volume of distribution of total drug after sodium valproate ($r = 0.94$, $P < 0.02$).

Figure 5 Relationship between change in free diazepam fraction and percent increment in plasma clearance after sodium valproate ($r = 0.94$, $P < 0.02$).

Table 2 Pharmacokinetic parameters calculated from total serum diazepam concentration following a single intravenous injection 10 mg in absence (a) and presence (b) of valproic acid.

Subject	Age (years)	Weight (kg)	$AUC_{0-\infty}$ ($\mu\text{g ml}^{-1}\text{h}$)	A (ng ml^{-1})	α (h^{-1})	$T_{1/2}\alpha$ (h)	B (ng ml^{-1})	β (h^{-1})	$T_{1/2}\beta$ (h)	Vd (L kg^{-1})	Cl (L h^{-1})
1	21	70	a 8271	1264	3.98	0.07	260	0.03864	17.9	0.45	1.21
			b 6718	652	2.67	0.26	234	0.04100	16.9	0.52	1.49
2	22	68	a 12801	1095	3.17	0.22	318	0.02684	25.8	0.43	0.78
			b 9084	771	1.89	0.37	204	0.02541	27.3	0.63	1.10
3	26	64	a 7338	1052	1.51	0.46	150	0.02531	27.4	0.83	1.36
			b 4584	1203	2.56	0.27	82	0.02584	26.8	1.32	2.18
4	26	75	a 6482	927	3.39	0.20	106	0.01586	43.7	1.29	1.54
			b 5080	830	2.63	0.26	53	0.01436	48.3	1.83	1.97
5	30	73	a 12927	1731	3.65	0.19	131	0.01230	36.4	0.86	0.77
			b 7923	795	1.10	0.63	91	0.01313	32.8	1.32	1.26
6	35	75	a 6536	1181	3.48	0.20	132	0.02102	33.0	0.96	1.53
			b 4068	888	3.36	0.21	83	0.02330	29.9	1.41	2.46
Mean	27	71	a 9059	1208	3.20	0.24	183	0.02332	34.0	0.80	1.20
s.d.	± 5	± 4	± 3018	± 280	± 0.87	± 0.11	± 86	± 0.00931	± 14.0	± 0.33	± 0.35
Mean			b 6243	857	2.37	0.33	125	0.02384	33.7	1.17	1.74
s.d.			± 1998	± 187	± 0.77	± 0.15	± 74	± 0.01006	± 14.0	± 0.50	± 0.54
			$P < 0.01$	$P < 0.01$	NS	NS	$P < 0.01$	NS	NS	$P < 0.05$	$P < 0.01$

Table 3 Pharmacokinetic parameters calculated from free serum diazepam concentration following intravenous administration 10 mg in absence (a) and presence (b) of valproic acid.

Subject		$AUC_{0-\infty}$ ($\mu\text{g ml}^{-1}\text{h}$)	$T_{1/2}\beta$ (h)	Vd (l kg^{-1})	Cl (l h^{-1})
1	a	137	18.7	28.0	72.8
	b	155	20.4	27.0	64.4
2	a	219	25.3	24.4	45.7
	b	312	27.5	18.6	32.1
3	a	132	26.9	46.1	75.8
	b	175	32.8	42.3	57.1
4	a	118	41.8	68.3	84.7
	b	155	45.6	57.1	64.9
5	a	223	54.2	43.0	44.8
	b	332	59.8	36.3	30.0
6	a	122	31.5	49.7	81.9
	b	187	34.9	36.0	53.5
Mean	a	158	33.1	44.1	67.6
s.d.		± 49	± 12.9	± 16.0	± 17.8
Mean	b	219	36.8	36.2	50.0
s.d.		± 81	± 14.0	± 13.2	± 15.6
		$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$

Table 3 summarises the kinetic parameters calculated from the unbound serum diazepam concentrations in each of the subjects studied. Half-lives ($T_{1/2}\beta$) calculated from the unbound serum diazepam concentration-time profile in the absence of valproic acid, did not differ significantly from those calculated from the total serum diazepam concentration-time profile. The $AUC_{0-\infty}$ was significantly higher and the apparent volume of distribution and intrinsic clearance of free drug were significantly lower in the presence of valproic acid.

Discussion

The binding of diazepam to serum proteins, has been determined using ultrafiltration at room temperature. Monks (1978) showed that the binding of phenytoin and valproic acid assessed by this method at room temperature is in good agreement with values obtained by equilibrium dialysis at 37°C. In fact during the centrifugation process the temperature inside the centrifuge varied between 28–30°C and this may also explain the good agreement found between free diazepam fraction determined by this method and data presented by previous authors (Klotz *et al.*, 1975; Kangas *et al.*, 1976; Greenblatt *et al.*, 1980) using equilibrium dialysis at 37°C. The calculated serum unbound diazepam concentration estimated from the values for free fraction and total serum

diazepam concentration therefore provide a reasonable assessment of the changes in free diazepam levels in serum caused by valproic acid.

In this study, the free fraction of diazepam was almost doubled in the presence of therapeutic serum concentrations of valproic acid. This was accompanied by a significant increase in apparent volume of distribution and systemic clearance of total drug, while the elimination half-life was unchanged. These findings would be consistent with displacement of diazepam from plasma protein binding sites by valproic acid.

Diazepam is a drug which is subject to restrictive (flow independent) elimination (Klotz *et al.*, 1976). For such a drug it has been shown that the total metabolic clearance (Cl) is directly proportional to the free fraction of the drug in blood (Shand *et al.*, 1975):

$$Cl = f Cl_i$$

where Cl_i or intrinsic clearance is a term directly related to the activity of the drug metabolising enzymes. In theory the displaced drug would not remain for long in the vascular compartment but would initially redistribute and subsequently be cleared by hepatic metabolism until steady state had been restored to the original free drug concentration, although at a lower total serum concentration. Although a slight and transient rise in free concentration might be expected when the bound diazepam

is displaced, this would be very small (less than 1% following redistribution) because the drug has a relatively large apparent volume of distribution.

In fact, a significant elevation of free diazepam concentration was found in our subjects when they were receiving concurrent sodium valproate. Furthermore, although the free fraction was almost doubled (by $93 \pm 34\%$), the corresponding increase in clearance was only $45 \pm 16\%$. The free intrinsic drug clearance was, however, reduced in the presence of valproic acid. These observations may suggest that valproic acid was inhibiting the metabolism of diazepam. This possibility is given weight by the fact that *N*-desmethyldiazepam concentrations were significantly reduced in the presence of valproic acid, although displacement of the metabolite from plasma protein binding sites could be an alternative explanation of this reduction, since it is 98% bound to plasma proteins (Van der Kleijn *et al.*, 1971).

A protein binding interaction between valproic

acid and phenytoin has been shown *in vitro* (Cramer & Mattson 1979; Monks & Richens 1980) and *in vivo* (Mattson *et al.*, 1978; Dahlqvist *et al.*, 1979; Frigo *et al.*, 1979). More recently valproic acid has been shown to inhibit the metabolism of phenobarbitone, phenytoin and possibly ethosuximide (Wilder *et al.*, 1978; Bruni *et al.*, 1980; Perucca *et al.*, 1980; Mattson & Cramer, 1980) and this would further support the possibility of an inhibitory effect on diazepam metabolism.

Our results may therefore be explained by a combination of a protein binding interaction and inhibition of metabolism. The further possibility of a displacement from tissue binding sites is also raised by the finding of a decreased apparent volume of distribution of free drug.

We thank the National Fund for Research into Crippling Diseases for financial support and Dr Emilio Perucca for helpful discussion.

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(Received July 10, 1981)