[Chem. Pharm. Bull. 36(7)2551—2556(1988)]

Diurnal Variation in Pharmacokinetics of Valproic Acid with Unequal Dosing Intervals

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(Received October 28, 1987)

The diurnal variation in pharmacokinetics of valproic acid (VPA) was studied at unequal dosing intervals, because patients generally take drugs with each meal or with poor compliance. Six healthy volunteers (22 to 32 years old) orally took 300 mg of sodium valproate at 9:00 and 18:00 each day for 6 d. On the sixth day, blood was drawn periodically for 24 h for pharmacokinetic analysis. Faster absorption and a shorter absorption lag-time were observed at morning dosing after a light meal, and this may be meal-related. The systemic clearance and the mean plasma level during the day were in good agreement with those during the night, although VPA plasma levels showed large diurnal fluctuations. Plasma albumin concentration at night was significantly decreased and this seems consistent with the finding that the free fraction (FF) of VPA was higher during the night. A significant negative correlation between plasma albumin concentration and the FF was observed. These results suggest that the VPA dosing schedule used here is adequate for maintaining the mean plasma level in an appropriate range. Mealtimes and blood sampling times should be taken into account in routine plasma VPA level monitoring to minimize the influence of diurnal variation.

Keywords——valproic acid; diurnal variation; pharmacokinetics; unequal dosing interval; protein binding

Since valproic acid (VPA) has a low extraction ratio in man, its clearance is dependent upon its free fraction. A large intraindividual variation in the free fraction was observed and this fluctuation could not be explained only in terms of its concentration-dependent protein binding.[1] Diurnal oscillations of the free fraction and the clearance have also been reported.[2-4] These findings should be taken into account to adjust the dosing schedule of VPA.

In general, drugs are taken after each meal because of the decrease of gastrointestinal side-effects or to minimize poor compliance and so on. VPA is usually prescribed to be taken with each meal. The antiepileptic drug should theoretically be dosed at least three, and preferably four, times a day because of its short half-life. However, two or three times-a-day dosing is the general rule.[5] Therefore, pharmacokinetic studies of VPA under more practical conditions, such as unequal dosing intervals, are necessary to discuss the diurnal variations in more detail.

Our aim was to document the circadian rhythms in total and free VPA pharmacokinetics in volunteers receiving two doses per day with unequal dosing intervals.

Experimental

Study Protocol——Our subjects were six male volunteers, and their ages ranged from 22 to 32 years with the mean of 24.3. The body weight in kg was between 47 and 68 and the average was 61.0. They were healthy with normal

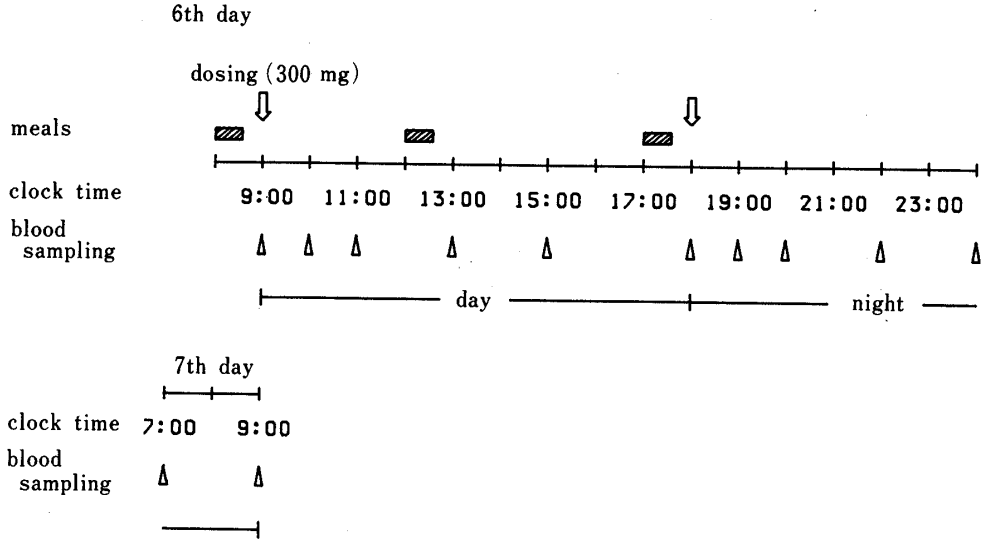

Fig. 1. Experimental Protocol during the Day of the Study

liver and kidney functions (screened by using routine clinical laboratory tests). The participants did not take any medication for at least two weeks prior to and during the day of the study. The volunteers were given two different VPA tablet preparations [sodium valproate tablet, 100 mg (lot No. 041 ADB) and 200 mg (lot No. 757 ADD) of Depakene®, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan] at a dose of 300 mg (260.3 mg as VPA) twice a day (at 9:00 and 18:00) for 6 consecutive days. Thus, the VPA dosing schedule was at equal doses and unequal dosing intervals.

The experimental protocol during the day of the study is illustrated in Fig. 1. In the calculation of pharmacokinetic parameters, plasma level of VPA at 18:00 as shown in Fig. 1 was used for both day and night. The meal time of this protocol was adjusted with that of inpatients in our hospital. Blood samples of about 5 ml were collected from an arm vein using a disposable syringe (Terumo Co., Ltd., Tokyo, Japan) and were immediately transferred into a vacuum blood collection tube containing ethylenediaminetetraacetic acid dipotassium salt as an anticoagulant. After centrifugation, a portion of the plasma obtained was used as soon as possible for the assay of free fatty acids (FFA). For drug assay and protein binding study, the rest of the plasma were frozen at $-40\,^{\circ}C$ and used within one week. Physical activity and food were not limited, but on the sixth day subjects had similar breakfast (200 ml of milk, a piece of bread, and eggs scrambled or boiled as requested) and dinner (a breaded pork cutlet or beef on rice in a deep bowl and a cup of vegetable soup). These were just like standard hospital meals.

Protein Binding——Protein binding of VPA was evaluated by ultrafiltration. The device used was the EMIT® FreeLevel$_{TM}$ System 1 (lot No. 6B549-R01 and -R01C, Syva Co., Palo Alto, CA, U.S.A.).[6] About 1 ml of plasma was poured into the reservoir and centrifuged at $2000 \times g$ (Hitachi centrifuge SCR-20B, Hitachi Koki Co., Ltd., Tokyo, Japan) for 15 min at $25 \pm 2\,^{\circ}C$. The degree of protein binding was calculated as the ratio of drug in the ultrafiltrate to that in plasma and expressed as the free fraction (FF, %).

Analyses——VPA in plasma and ultrafiltrate was analyzed by a modified method of gas-liquid chromatography,[7] originally developed by Levy and others.[8] The plasma albumin concentrations were measured by the bromcresol green method[9] using an IATRON albumin kit (IATRON Laboratories, Inc., Tokyo, Japan). Plasma FFA levels were determined with a NEFA kit-U (Nippon Shoji Kaisha, Ltd., Osaka, Japan) applying enzymatic analyses based on the method of Mizuno *et al.*[10]

Calculations——The pharmacokinetic parameters of VPA were analyzed in terms of a one-compartment open model with first-order absorption and obtained by using a personal computer program, MULTI,[11] including our developed subroutine program for unequal doses and dosing intervals.[12] The parameters were the absorption lag-time, the apparent first-order absorption rate constant, k_a, the apparent first-order elimination rate constant, K, and the apparent volume of distribution, V_d. The personal computer used was an FM-11EX (Fujitsu, Tokyo, Japan). Systemic clearance, Cl_{sys}, of VPA at unequal dosing intervals was calculated as the product of K and V_d.[13] Mean total ($\bar{C}$) or free ($\bar{C}_f$) plasma level was obtained by dividing AUC, which is the area under the total or free plasma level–time curve determined by means of the trapezoidal rule assuming complete bioavailability[14,15] by dosing intervals in hours. The time required for the maximum plasma VPA level (C_{max}) at each dosing, t_p, C_{min} (which is the minimum concentration in the elimination phase), and C_{max} were obtained from the simulation of the drug plasma level–time curve by using the resultant pharmacokinetic parameters.[12] A calculation of $(C_{max(n)} - C_{min(n-1)})$ was performed and the resultant was assessed as one of the absorption characteristics.

Statistical analyses were performed by using the paired t-test and Wilcoxon signed rank test. A p value of less

than 0.05 was considered to be significant.

Results

A typical observed time course of VPA plasma levels is illustrated in Fig. 2. This shows that the absorption started quickly and was faster after the morning dose. The pharmacokinetic parameters and rates of bioavailability for VPA in the day and at night are compared in Table I. The absorption lag-time and t_p were increased by 86.0 and 98.8%, respectively and a 70.7% decrease in k_a was found during the night, although these were not statistically significant (paired t-test). The value of $(C_{max(n)}-C_{min(n-1)})$ in the day was significantly ($p<0.025$) larger. The systemic clearance and the mean plasma level during the day were statistically in good agreement with those during the night.

In Table II, the pharmacokinetic parameters of free VPA, $\bar{C}_f$ and FF, plasma albumin and FFA concentrations are presented. The FF during the night was 24.5% higher, but there was no significant difference between day and night (paired t-test). Plasma albumin concentration during the nighttime was significantly decreased (Wilcoxon signed rank test, $p=0.0156$). This may support the finding that the FF was higher during the night.

Based on these observations, the correlation between plasma albumin concentration and

TABLE I. Pharmacokinetic Parameters of VPA in Normal Human

Subject	k_a (h^{-1})		Absorption lag-time (h)		t_p (h)		$C_{max(n)}-C_{min(n-1)}$ (mg/l)		$\bar{C}$ (mg/l)		Cl_{sys} (ml/h/kg)		Cl_{int} (ml/h/kg)	
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
1	8.20	8.17	0.90	1.95	1.44	2.48	25.4	24.0	51.7	59.9	10.24	9.02	95.2	104.2
2	16.79	0.12	0.00	0.49	0.30	5.12	27.1	9.2	40.0	36.7	8.40	9.52	160.9	206.1
3	8.87	2.51	0.89	−0.09	1.38	1.13	30.5	23.5	41.6	35.9	8.36	9.59	127.2	75.9
4	2.07	0.78	0.78	0.64	2.25	3.18	19.3	16.6	36.3	35.7	8.77	8.80	139.9	134.8
5	0.50	0.95	−0.44	0.79	2.85	3.12	33.2	21.6	54.3	52.8	6.50	6.32	101.7	97.5
6	9.46	0.89	0.88	1.80	1.34	4.07	31.8	19.2	34.4	30.9	10.82	12.17	112.9	72.1
Mean±	7.65	2.24	0.50	0.93	1.60	3.18	27.9[a]	19.0[a]	43.1	42.0	8.85	9.24	123.0	115.1
S.E.M.	2.39	1.23	0.24	0.32	0.36	0.56	2.1	2.3	3.3	4.7	0.63	0.76	10.1	20.4

Statistical comparisons between day and night were performed by using the paired t-test. *a*) $p<0.025$.

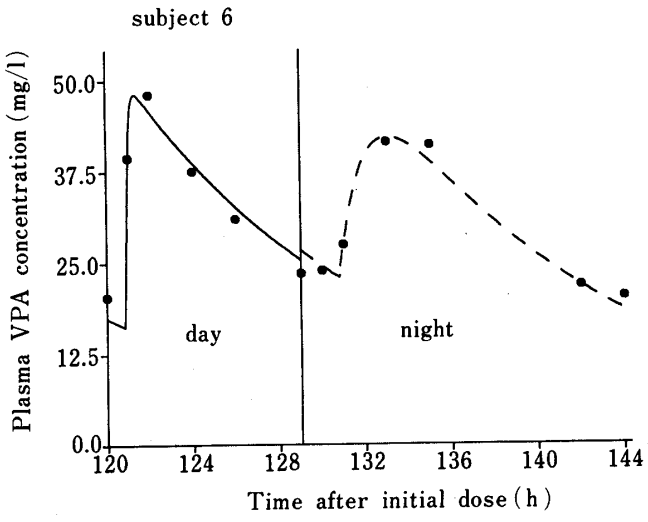

Fig. 2. Time Course Observations of VPA Plasma Levels

The solid line shows the best fitting simulation during the day and the broken line shows that during the night. Each point is an observed level in subject 6.

TABLE II. Mean Free Level and Free Fraction of VPA, Plasma Albumin and FFA Concentrations in Normal Human

Subject	$\bar{C}_f$[a] (mg/l)		FF[a] (%)		Albumin level[b] (g/100 ml)		FFA level[b] (μeq/l)	
	Day	Night	Day ($n=6$)	Night ($n=7$)	Day ($n=6$)	Night ($n=7$)	Day ($n=6$)	Night ($n=7$)
1	5.63	5.07	10.76±0.81	8.66±0.46	5.13±0.10	4.99±0.16	149.0±37.1	336.8±59.0
2	2.24	1.81	5.22±0.62	4.62±0.30	5.37±0.02	5.15±0.16	209.0±43.3	175.8±25.4
3	2.73	4.59	6.57±0.21	12.64±1.81	4.98±0.42	4.76±0.41	216.8±26.9	183.4±36.7
4	2.26	2.43	6.27±0.18	6.53±0.39	5.07±0.43	5.00±0.34	279.1±26.3	259.5±38.1
5	3.50	3.53	6.39±0.39	6.48±0.42	5.53±0.21	5.47±0.15	316.7±50.2	183.8±21.3
6	3.12	4.54	9.58±2.45	16.87±3.75	5.23±0.14	4.17±0.32	185.2±20.9	142.6±17.0
Mean±	3.25	3.66	7.47[d]	9.30[d]	5.22[c,d]	4.92[c,d]	226.0[d]	213.7[d]
S.E.M.	0.52	0.53	0.89	1.88	0.08	0.18	25.2	29.2

The values of FF of VPA and albumin or FFA level are each the mean±S.E.M. *a, b*) Statistical comparisons between day and night were made by using the paired *t*-test (*a*) and the Wilcoxon signed rank test (*b*). *c*) $p<0.02$. *d*) These were calculated by using the mean value of each subject.

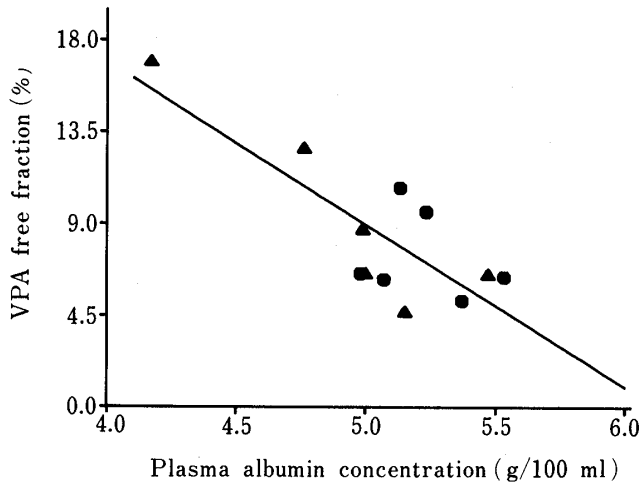

Fig. 3. Correlation between Plasma Albumin Concentration and VPA Free Fraction

The solid straight line represents the regression line calculated with the least-squares method; the equation was $y=-7.93x+48.6$. ■, day; ▲, night.

the FF was examined and the results obtained are illustrated in Fig. 3. A significant negative correlation was obtained ($n=12$, $r=-0.799$, $p<0.01$). A significant negative correlation between plasma albumin concentration and Cl_{sys} was also found ($n=12$, $r=-0.762$, $p<0.01$). However, there was no correlation between the FF ratio of night to day and the albumin concentration ratio of day to night.

On the other hand, the effect of FFA, which has been accepted to affect the protein binding of VPA,[16-18] on the FF was studied. Although the plasma FFA levels were slightly decreased by 5% during the night, the FF was increased by more than 20%. These findings suggest that the effect of changes in the FFA level on the FF was negligible in this study.

Discussion

The rate, but not the extent, of VPA absorption is markedly affected by meals. Chun *et al.* have shown[19] that the time required to reach the peak serum concentration after oral administration of VPA was shorter on fasting than immediately after meals. As can be seen in Table I, the absorption lag-time and t_p during the night were about twofold larger than those during the day, although these differences were not statistically significant. Moreover, k_a and

($C_{max(n)} - C_{min(n-1)}$) were larger during the daytime than during the nighttime and the latter difference was significant (paired t-test). These results could be meal-related, because the breakfast on the study day was a light meal and the dinner was of a normal size.

Concerning the free concentration of VPA, Bauer and others have found[3,4] that the level was significantly higher in the day, and their observation was opposite to ours. One of the reasons may be the VPA dosing schedule: their volunteers were fasted overnight before the morning dosing and the fasting increased FFA levels,[20] which could increase FF of VPA.[16-18] On the other hand, Patel *et al.* have reported[2] that the maximum free fraction of VPA was observed between 2 and 6 a.m. in 7 out of 10 cases, and this is consistent with our findings.

VPA is a low-clearance drug nearly 100% metabolized by the liver. Because of this, the hepatic clearance, Cl_h, and the intrinsic clearance, Cl_{int}, are related as follows:

$$Cl_{sys} \simeq Cl_h = (FF/100) \times Cl_{int}$$

The Cl_{int} in ml/h/kg calculated from the values of Cl_{sys} (Table I) and FF (Table II) is also shown in Table I. The Cl_{int} during the night was decreased in two volunteers (subjects 3 and 6), and one (subject 2) showed the opposite phenomenon. The rest showed no diurnal change, and a large interindividual variation in the Cl_{int} was found. VPA metabolism in man mainly involves β-, ω_1-, and ω_2-oxidation and glucuronidation.[21] In nocturnal animals such as the rat, the maximum metabolism of drugs by $9000 \times g$ supernatant fractions has been found at around midnight.[22] The change in drug metabolism by the fractions corresponds to a change in the level of plasma corticosterone.[23] Peak corticosteroid concentrations are seen in the early morning hours in human subjects and in early evening in nocturnal animals.[24] On the other hand, uridine diphosphate (UDP)-glucuronosyltransferase activity in the rat was higher in the evening.[25] The urinary excretion of glucuronide and oxidation products for VPA varies substantially in humans.[21] These findings suggest that VPA metabolism may be affected by various factors, and therefore, we may also have to consider the metabolic characteristics for VPA in subjects to clarify the causes of the large interindividual variations in Cl_{int} between day and night.

Plasma albumin concentrations during the night were significantly lower, in agreement with the finding of Touitou *et al.*[26] They reported that the lowest plasma protein concentrations were found around 4:00 a.m. and the highest between 8:00 a.m. and noon. Although there was no significant difference between day and night in the FF of VPA, the FF increased with decreasing albumin concentration. A significant negative correlation between plasma albumin level and the FF of VPA was found, as illustrated in Fig. 3. Also, a significant negative correlation between plasma albumin concentration and the Cl_{sys} of VPA was found. Our observations suggest that plasma albumin levels affect the disposition of VPA. However, there was no correlation between the changes of FF and albumin concentration during the night. Therefore, we will have to examine the cause of these diurnal variations in more detail.

FFA concentrations in plasma play an important role in the protein binding of VPA.[2,18,27] No significant differences in FFA levels between day and night were observed in this report. Patel *et al.* have found[2] a significant positive correlation between FFA level and valproate free fraction, while there was no significant correlation in our study. Robinson and others have recently reported[28] that FFA concentration did not have a significant effect on the free fraction of VPA. Although the reasons for the discrepancy are not apparent, one possibility may be that albumin level influences VPA protein binding.

As shown in Fig. 2, VPA plasma levels showed large diurnal fluctuations. Bauer and others have found[3,4] that the daily variation of plasma VPA concentrations is large. Therefore, sampling may best be carried out just prior to the next dosing for routine plasma VPA level monitoring because the effects of drug absorption characteristics are minimum. On

the other hand, the VPA dosing schedule used here is an adequate one, since the Cl_{sys} and $\bar{C}$ during the day were in good agreement with those during the night.

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