Time-Dependent Kinetics III: Diurnal Oscillations in Steady-State Plasma Valproic Acid Levels in Rhesus Monkeys

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Abstract □ Valproic acid was administered by constant rate intravenous infusion to catheterized chaired rhesus monkeys for 8–10 weeks under controlled environmental conditions. Steady-state plasma levels were monitored at 2-hr intervals for 26 hr (10 am–12 noon on the following day), 1 day/week for 6 weeks. Individual steady-state plasma concentration–time plots exhibited the following characteristics. During Period A (10 am–6 pm), plasma levels remained stable or decreased. During Period B (6 pm–6 am), plasma levels increased, reached a maximum, and remained markedly higher than during Period A. The maximum concentrations were 40–140% higher than the observed minimum concentrations. During Period C (6 am–noon), plasma levels tended to decline from the maximum concentrations achieved in Period B. In most cases, plasma concentrations at 10 am and 12 noon of the 2nd experimental day fell within 10% of their respective values on the previous day. The mean (±*SD*) periods obtained by cross-correlation analysis of individual plasma concentration–time plots were 30.7 (±3.7) and 22.8 (±3.6) hr for Animals 903 and 923, respectively. The corresponding mean (±*SD*) amplitudes were 27.3 (±12.6) and 17.4 (±2.3)%. A circadian rhythm in total body clearance was hypothesized, and its pharmacokinetic implications are discussed.

Keyphrases □ Valproic acid—steady-state plasma levels, time-dependent kinetics, monkeys □ Kinetics, time dependent—valproic acid, steady-state plasma levels, monkeys □ Pharmacokinetics, time dependent—valproic acid, steady-state plasma levels, monkeys □ Anticonvulsants—valproic acid, steady-state plasma levels, time-dependent kinetics, monkeys □ Circadian rhythm—valproic acid, steady-state plasma levels, time-dependent kinetics, monkeys

Preliminary studies dealt with the pharmacokinetic properties of valproic acid (dipropylacetic acid) following single-dose administration at several dose levels to six male rhesus monkeys (1). These studies were undertaken to determine appropriate dosage regimens of the drug in view of its efficacy testing in an epileptic monkey model (2, 3). Analysis of single-dose data revealed that: (*a*) the pharmacokinetic behavior of valproic acid could be described adequately by a one-compartment open model, (*b*) the total body clearance was 0.19 liter/hr/kg and dose independent, and (*c*) the elimination half-life was 0.7 hr.

The short biological half-life of valproic acid suggested that a discontinuous mode of administration such as multiple oral dosing would be inappropriate since the frequency of administration required to obtain less than 50% oscillations in plasma levels would not be practical. A continuous mode of administration such as constant rate chronic intravenous infusion was then considered.

Monitoring of steady-state plasma levels during constant rate zero-order infusion of the drug to monkeys revealed noticeable fluctuations in plasma levels. Diurnal oscillations in steady-state plasma ethosuximide levels were previously observed under comparable experimental conditions (4, 5). A similar phenomenon was suspected for valproic acid, and it was investigated further under controlled experimental conditions.

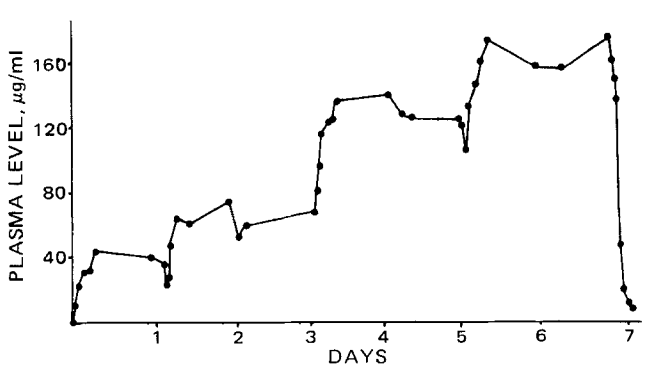

Figure 1—*Four-stage infusion of valproic acid to Animal 913.*

EXPERIMENTAL

Three chronically catheterized male rhesus monkeys (733, 903, and 923) with implanted electrodes in the skull were used. Animals 903 and 923 had been used previously to investigate the diurnal variations in steady-state plasma ethosuximide levels (5). The animals received continuous zero-order infusions of valproic acid over 8–10 weeks (sterile valproic acid solutions in normal saline; infusion rates of 30–40 mg/hr yielding steady-state plasma levels of 30–55 μg/ml).

Plasma samples of 0.5 ml were collected 1 day/week at 2-hr intervals over 26 hr for 6 consecutive weeks. Plasma fractions, 0.2 ml, were assayed for valproic acid in duplicate using a previously described GLC procedure (6). Electroencephalogram (EEG) data were collected by continuous EEG monitoring (10 am–10 am or 10 pm–6 am) with or without blood sampling. Only 1 week of data could be obtained for Animal 733 because of technical difficulties with catheters.

The other experimental conditions used in the present study were similar to those described previously (5). These included: housing of animals in an isolated room with diurnal lighting (light during day, 6 am–6 pm; and dark during night, 6 pm–6 am); strict feeding protocol (6 am, monkey chow; and 5:30 pm, monkey chow and fresh fruits) and noise schedule (moderately high volume, 6 am–6 pm; and low volume, 6 pm–6 am); infusion of drug solutions; blood sampling; and EEG monitoring from an adjacent room.

Periodic analysis of the data was performed using the cross-correlation technique (7) as reported earlier (5).

RESULTS

Preliminary Observations—Previous studies[1] in these laboratories involved single-stage and multistage constant rate infusions of valproic acid to four monkeys to achieve steady-state plasma levels between 20 and 150 μg/ml. An example of the data collected is shown in Fig. 1. Because of its short elimination half-life, steady-state levels of valproic acid were achieved within a few hours. However, appreciable variations in steady-state plasma levels were apparent in each stage. The difference between maximum and minimum steady-state plasma concentrations ranged from 10 to 50%. Similar variations in steady-state plasma levels were also noticed in other animals.

Fluctuations in steady-state plasma levels could be related to variations in experimental factors such as drug assay and/or infusion pump. The GLC assay precision was 6% or better and could not totally explain the observed fluctuations (6). Previously (5), the delivery rate of infusion

[1] Unpublished data.

Table I—Oscillations in Steady-State Plasma Levels of Valproic Acid during Six Sampling Periods

Animal	Week	C_{max}, μg/ml	C_{min}, μg/ml
733	5	50.1	28.2
903	1	44.8	30.1
	2	48.0	23.9
	3	44.0	31.2
	4	56.2	31.2
	5	53.5	35.3
	6	59.5	28.6
923	1	44.1	18.5
	2	35.3	21.1
	3[a]	—	—
	4	37.4	25.4
	5	42.6	26.3
	6	41.3	25.7

[a] Plasma samples were not collected because of technical difficulties with the infusion catheter.

pumps was shown to be relatively constant over several 24-hr periods. These considerations suggested that the observed fluctuations in steady-state plasma valproic acid levels represent a real phenomenon. This phenomenon could possibly be related to diurnal variations in total body clearance, as was observed for ethosuximide (5). To investigate this hypothesis further, additional studies were carried out under controlled conditions.

EEG and Plasma Concentration–Time Data—The sleeping behavior of animals was established by EEG monitoring. Animals were awake during most of the day periods and were in various stages of sleep during the night periods. These data are consistent with those obtained previously (5).

Figures 2A and 3A show typical diurnal fluctuations in steady-state plasma levels observed over a given sampling period (Week 2) for Animals 903 and 923, respectively, and Figs. 2B and 3B represent the corresponding average diurnal oscillations over six sampling periods. Plasma levels were not constant but fluctuated appreciably over the sampling period. The following characteristics were present in individual steady-state plasma concentration–time plots:

Period A (10 am–6 pm)—Plasma levels remained stable (Animal 903, Weeks 1, 3, and 5; and Animal 923, Weeks 1, 2, and 6) or decreased (Animal 733, Week 5; Animal 903, Week 4; and Animal 923, Week 4).

Period B (6 pm–6 am)—In all cases, plasma levels increased and remained markedly higher than those observed during Period A. The C_{max} (highest concentration during the 26-hr observation period) was always reached during this period: between 2 am and 6 am for Animal 903 and between midnight and 6 am for Animal 923. The C_{max} values were generally 40–140% higher than the corresponding C_{min} values (lowest concentration during the 26-hr observation period) (Table I).

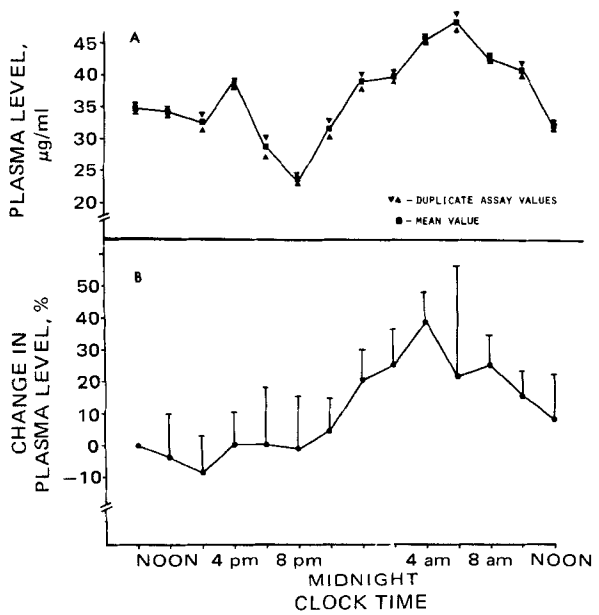

Figure 2—*Plots of typical diurnal changes in steady-state plasma levels during a given sampling period (A) and mean (±SD) percent changes in plasma levels* versus *time (B) for Animal 903.*

Table II—Cross-Correlation Analysis of Experimental Data

Animal	Week	Period, hr	Amplitude, %
733	5	21.0	17.24
903	1	26.0	16.69
	2	33.0	46.77
	3[a]	—	—
	4	34.5	29.51
	5	27.5	15.75
	6	32.5	27.78
	Mean ± *SD*	30.7 ± 3.7	27.30 ± 12.55
	Mean profile	31.5	17.92
923	1	22.0	20.07
	2	17.0	17.48
	3[b]	—	—
	4	25.0	15.96
	5	24.0	19.09
	6	26.0	14.34
	Mean ± *SD*	22.8 ± 3.6	17.39 ± 2.31
	Mean profile	25.5	14.41

[a] The cross-correlation analysis failed to give meaningful estimates of period and amplitude. [b] Plasma samples were not collected because of technical difficulties with the infusion catheter.

Period C (6 am–Noon)—As in Period B, the behavior of plasma levels was quite consistent within and between animals. Plasma levels tended to decline from the C_{max} achieved during Period B. In most cases, plasma concentrations at 10 am and 12 noon of the 2nd experimental day fell within 10% of their respective values on the previous day.

In a previous study (5), application of the cross-correlation analysis (7) to experimental data obtained using a similar protocol was evaluated and was satisfactory. In the present study, sets of individual plasma concentration–time data as well as the mean set of data for each animal were analyzed with that technique to obtain estimates of period and amplitude (Table II). The mean (±*SD*) periods were 30.7 (±3.7) and 22.8 (±3.6) hr for Animals 903 and 923, respectively. The corresponding periods obtained from mean profiles (Figs. 2B and 3B) were 31.5 and 25.5 hr. These values suggest the possibility of a circadian rhythm in steady-state plasma levels. The estimated mean (±*SD*) amplitudes were relatively large, 27.3 (±12.6) and 17.7 (±2.3)% for Animals 903 and 923, respectively.

DISCUSSION

Variations in experimental factors such as GLC assay and/or infusion pump could not explain the diurnal oscillations in steady-state plasma levels observed in the present study. The latter could be related to a circadian rhythm in total body clearance (volume of distribution, renal

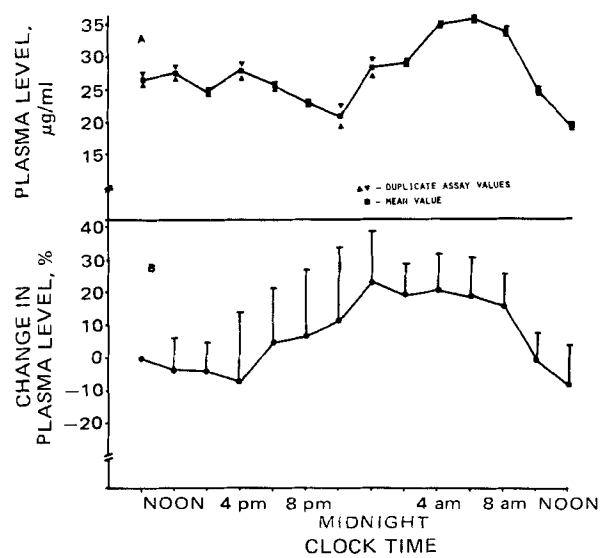

Figure 3—*Plots of typical diurnal changes in steady-state plasma levels during a given sampling period (A) and mean (±SD) percent changes in plasma levels* versus *time (B) for Animal 923.*

excretion, and/or metabolism), as was suggested for another acidic anticonvulsant, ethosuximide (5).

Valproic acid is highly bound to plasma proteins in monkeys ($\beta = 0.9$ at 100 μg/ml). Free fatty acids in plasma are highly protein bound, and they have been reported to displace clofibrate, a highly protein-bound hypolipidemic agent, from albumin binding sites (8). Crouthamel (9) suggested that these observations may have widespread significance, particularly since free fatty acid concentrations fluctuate widely. Concentrations of blood free fatty acids are markedly influenced by such stimuli as exercise, fasting, emotional stress, insulin, and glucose and by such clinical conditions as diabetes, infection, hypothyroidism, hemodialysis, and adrenergic stimulation (9, 10), Thus, the observed oscillations in plasma levels of valproic acid may possibly be related to fluctuations in free fatty acid levels and competitive displacement from protein binding sites.

An appreciable fraction of valproic acid (5–30%) is excreted unchanged in urine in monkeys (1). Diurnal variations in excretion rate, possibly related to a diurnal urinary pH rhythm, were reported in humans for sulfasymazine (11), salicylate (12), and amphetamine (13). It is not known whether a circadian rhythm in uninary pH exists in monkeys. However, a rhythm in the excretion rate of valproic acid would require urinary pH values around 4.5 [pKa of valproic acid = 4.5 (14)].

Circadian variations in the rates of *in vitro* metabolism of drugs undergoing oxidative metabolic transformations (hexobarbital, aminopyrine, *p*-nitroanisole, and 4-dimethylaminoazobenzene) were reported (15–19). Valproic acid is metabolized to a significant extent (70–95%) in monkeys (1), and some metabolic pathways in rats involve oxidative processes (20, 21). Thus, circadian variations in the activities of hepatic drug-metabolizing enzymes, if present in monkeys, could contribute to the observed oscillations in steady-state plasma levels.

Diurnal variations in steady-state plasma ethosuximide levels in monkeys were observed previously under similar experimental conditions (5). Although ethosuximide and valproic acid are both weak acids, they differ from each other both structurally and pharmacokinetically. The total body clearance of valproic acid is 10 times larger than that of ethosuximide [195 *versus* 19.2 ml/hr/kg (1, 22)], and the elimination half-life of the former is one-fortieth that of the latter [0.7 *versus* 28.5 hr (1, 22)]. Moreover, valproic acid is highly protein bound whereas ethosuximide is only negligibly bound to plasma proteins (23, 24). The fact that diurnal oscillations are present for both drugs in spite of these differences leads one to speculate whether other drugs would also exhibit diurnal variations in steady-state plasma levels under similar conditions.

In linear pharmacokinetics (dose and time independent), zero-order intravenous infusion represents the most reliable method of maintaining stable steady-state drug levels. The present study shows that in the presence of time dependency, stable steady-state levels could not be maintained with a zero-order infusion approach. These and other experimental findings (5) clearly indicate the need for pharmacokinetic models with oscillatory disposition functions. Such models are not yet available. The need for new approaches to drug administration is also apparent.

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