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Time-Dependent Kinetics VIII: Absence of Diurnal Oscillations in Valproic Acid Disposition Following Single Dose Administration to Rhesus Monkey

Keyphrases □ Valproic acid—time-dependent kinetics, absence of diurnal oscillations, disposition following single dose administration to rhesus monkey □ Kinetics, time-dependent—absence of diurnal oscillations in valproic acid disposition following single dose administration to rhesus monkey

To the Editor:

Valproic acid exhibits an unusual pharmacokinetic property in the rhesus monkey, namely, extensive diurnal oscillations in systematic clearance. An initial study in three normal rhesus monkeys showed that during constant rate intravenous infusion, steady-state levels increased at night with maxima 40–140% higher than the corresponding minima (1). In a subsequent study in four normal monkeys, where levels were monitored for 48 hr, it was found that the diurnal fluctuations in steady-state levels were reproducible in 2 consecutive days (2). Furthermore, after reversal of the 12-hr light–12-hr dark cycle, plasma concentrations tended to follow the phase shift with maxima during the reversed dark phase (actual day time) (2). In a later efficacy study in 12 epileptic monkeys, valproic acid was infused at a constat rate to achieve steady-state levels of 46, 97, and 147 μg/ml (3). The diurnal fluctuations in valproate levels were found at all three steady-state concentrations (4).

In each of the studies just described, the diurnal changes in valproate clearance were found under steady-state conditions. In the present study, the objective was to determine whether this phenomenon could be observed with an acute mode of administration. To this effect, valproate was administered at different times of day by intravenous boluses to a group of six rhesus monkeys. In addition, this design would allow a detection of diurnal effects in valproate distribution.

Six chair-adapted male rhesus monkeys (mean body weight 4.1 kg) with two chronic venous catheters (femoral for valproate bolus injection and jugular for blood sampling) were used in this study. Environmental conditions were maintained the same as those described previously (1, 2) (diurnal cycle: light period, 6 am–6 pm; dark period, 6 pm–6 am). Based on the findings of previous studies (valproate plasma levels remained stable or decreased during 10 am–6 pm, increased and reached a maximum during 6 pm–6 am, and tended to decline from 6 am–noon), the following times were selected for drug administration: 2 am, 8 am, 2 pm, and 8 pm. At these times of the day, valproate intravenous bolus injections were administered in a randomized fashion to six monkeys. At least 1 week of rest was allowed between any two injection times. From 2 to 4 replicate studies at each time period were conducted for each of the six monkeys.

At each time period, including replications, each monkey received 63.75 mg of valproic acid equivalent as sodium salt in 0.5 ml of sterile saline as an intravenous bolus injection. An additional 5 ml of saline was used to flush the line after bolus administration. Blood samples (2 ml) were collected in vacuum tubes containing edetic acid at 2, 20, 40, 60, and 100 min following drug administration. Plasma was separated and frozen until assay. Valproate was assayed by GLC using the procedure of Levy *et al.* (5).

The area under the plasma concentration–time curve (extrapolated to infinite time) was calculated by the trapezoidal rule and the systemic or total body clearance was computed from the dose–area relationship. The plasma valproate concentration time data were least-squares fitted to a monoexponential decay equation (BMDX-85), and the volume of distribution was calculated by the ratio of total body clearance and elimination rate constant. The null hypothesis of equal mean values for clearance, volume of distribution, and elimination rate constant among treatments was tested using a one-way ANOVA for repeated measures (BMDP2V). Then Tukey's method for multiple comparisons (6) was used to test for differences between particular pairs of treatments.

Mean plasma valproate concentration–time profiles at 2 am, 8 am, 2 pm, and 8 pm are shown in Fig. 1. Based on the findings of previous studies, which revealed the presence of circadian rhythms in steady-state valproate plasma levels (1–4), lower clearance values would be expected at 2 am and 8 pm than at 2 pm and 8 am. However, no significant difference in valproate clearance was observed between any of the four time periods. Volume of distribution and half-life also did not exhibit any time dependence ($p > 0.05$). Values of clearance, volume of distribu-

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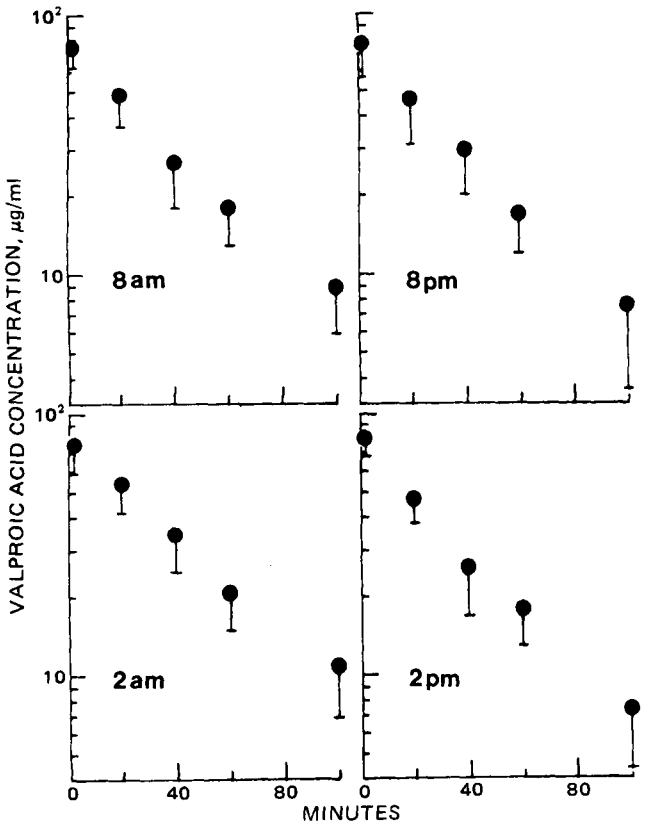

Figure 1—*Mean plasma valproate concentration–time profiles for six rhesus monkeys following four separate drug administrations by intravenous bolus injections.*

Table I—Pharmacokinetic Parameters Following Single Dose Administration of Valproic Acid to Rhesus Monkey

	2 am	8 am	2 pm	8 pm
Clearance, liter/hr^{-1} Mean (*SD*)	1.231 (0.369)	1.150 (0.267)	1.281 (0.390)	1.214 (0.264)
Volume of distribution, liter Mean (*SD*)	0.955 (0.263)	0.765 (0.080)	0.766 (.112)	0.795 (0.146)
Elimination Rate Constant, hour^{-1} Mean (*SD*)	1.312 (0.271)	1.517 (0.266)	1.636 (0.392)	1.573 (0.330)

tion, and half-life (Table I) are very similar to those found previously (1, 3, 7).

A consistent pattern of diurnal fluctuations in systemic clearance was previously found in three separate studies (1–4). The difference in experimental findings between those studies and the present one may be explained by the difference in the nature of the clearance obtained in both instances. In the former studies (1–4), valproate was administered by constant rate intravenous infusion and thus enabled the determination of an instantaneous systemic clearance at steady state. In the present study, however, valproate was administered by single bolus injection and resulted in the determination of an average clearance, a hybrid of the clearances operating during the elimination of the majority of the administered dose. Under steady-state conditions, differences in instantaneous clearance can be measured within a few hours and several (10–12) clearance determinations can be obtained in 24 hr. In contrast, the single dose approach revealed only four 2-hr hybrid samples of the diurnal evolution of drug clearance. Thus, in the case of valproic acid in the rhesus monkey, the single dose clearance represents an insensitive tool in the characterization of the time dependency in clearance.

Also, comparison of steady-state clearances between any two time points of the diurnal cycle involves only intraday variability in clearance. However, since each determination of single-dose clearance required several blood samples, any two clearance determinations (at the same time or at different times) were separated by at least a 1-week period. Appreciable interday variability was present. Table 1 shows that relative standard deviations of replicates at a given time of administration varied between 22 and 30%.

It may be argued that the particular group of six monkeys used in the present study does not exhibit the property of diurnal oscillations in steady-state clearance as was observed in previous studies (1–4). This possibility is eliminated by the fact that, in a later study (of diurnal fluctuations in cerebrospinal fluid valproate levels) four of the present six monkeys exhibited typical diurnal oscillations in steady-state plasma and cerebrospinal fluid levels (unpublished data). An alternative hypothesis to explain the findings of this study could be that chronic exposure to valproic acid is necessary to evoke a circadian rhythm in plasma concentration, *i.e.*, the constant presence of valproate *in vivo* would initiate an endogenous cycle in metabolism. This would be possible if, for example, chronic infusion (as opposed to bolus administration) of valproic acid was associated with depletion of cofactors necessary in the biotransformation of valproate. Such cofactors would then become rate-limiting and, if their synthesis or turnover rate was under diurnal control, valproate clearance would also exhibit a diurnal rhythm. In relation with this hypothesis, it is of interest to note that diurnal oscillations in steady-state levels have been observed with other drugs [ethosuximide (8), carbamazepine (9) and clonazepam (10)] in the rhesus monkey under the same experimental conditions. However, no studies of diurnal variation following single dose administration have been performed with these drugs. Therefore, several *in vivo* and *in vitro* metabolic studies will be necessary before the cofactor depletion hypothesis can be generalized.

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Centering Tool for Dissolution Vessels

Keyphrases □ Drug dissolution—testing, centering tool for dissolution vessels □ Centering tool—drug dissolution testing, for basket and paddle methods

To the Editor:

Proper alignment and standardization of equipment is essential to obtain reliable results in drug dissolution testing. The USP XX requires that the shaft in both the basket and paddle methods be positioned so that its axis is not >0.2 cm at any point from the vertical axis of the vessel (1). A centering tool for alignment of the shaft was previously described (2), and several centering tools are commercially available[1,2]. We have designed a centering tool which has been widely used in FDA laboratories and which we believe offers significant advantages over other designs.

The tool is machined from a block of plexiglass (Fig. 1). The sides of the tool are tapered at an angle of 30° from the axis so that the tool can be used with plastic or glass dissolution vessels or to align the base plates that support the dissolution vessels. The main slot is oversized to 10 mm to allow the tool to be used with 9.52-mm diameter shafts.

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[2] Van-Kel Industries, Chatham, NJ 17928.

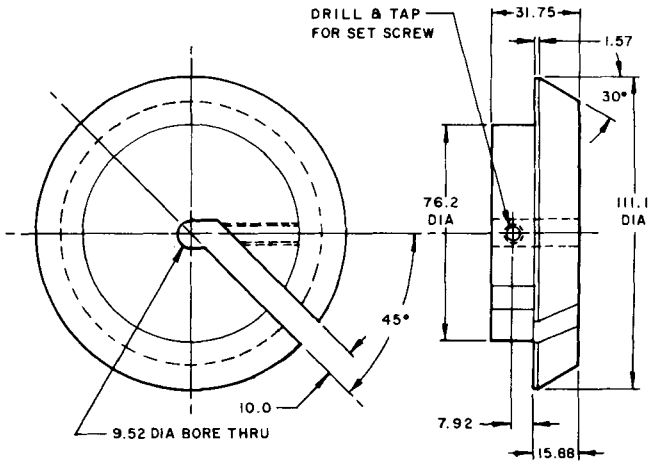

Figure 1—*Dissolution vessel centering tool. The material used was plexiglass and all measurements are in millimeters.*

The center portion of the slot is offset 45° from the main slot, so that the shaft can be accurately butted against the radius of the 9.50-mm center hole with a 6.35-mm screw. Securing the shaft against the center hole radius prevents the tool from accidental tipping when aligning the vessels. The final machining of the tapered edge is done with the device secured by its set screw to an arbor to ensure that the center hole and the tapered portion of the tool will be concentric.

While in use, the tool is slipped down the shaft into the mouth of the vessel, and the vessel is aligned with the tool after tightening the set screw. The design offers these advantages:

1. Locking the tool to the shaft prevents the alignment from changing while adjusting the kettle.
2. The circular design aligns the kettle's total circumference in one operation.
3. Dimension of the tool permits it to be used to align the base plate.

With proper use, most, if not all, of the centering tools previously described should be capable of aligning the shaft within USP specifications.

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