Chronopharmacological Study of Valproic Acid in Mice: Comparison of Oral and Rectal Administration

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(Received March 12, 1992)

This study was performed to investigate the influence of the dosing route on chronopharmacological aspect of valproic acid (VPA) in mice, comparing the oral and rectal route. ICR male mice, housed under a standardized light-dark cycle (lights on from 0700 to 1900), were orally or rectally administered 400 mg/kg VPA each at the following scheduled time: 0900, 1300, 1700, 2100, 0100 and 0500. VPA concentrations in plasma and brain were determined by gas-liquid chromatography. There was a circadian rhythm in the electroshock seizure (ES) threshold 30 min after oral VPA administration, with the highest value at the midnight (1300) and the lowest at the middark (0100) (p<0.01). A significant circadian rhythm was also found in plasma and brain VPA concentrations 30 min after oral administration (p<0.01). This finding is related to the rhythm in the ES threshold. In contrast to oral administration, no circadian rhythm in the ES threshold, plasma and brain VPA concentrations was observed after rectal administration. These values after rectal dosing showed higher levels in comparison to those after oral dosing. Thus, the rectal route for VPA might have merit to eliminate the time-dependent changes in VPA pharmacologic action and kinetics. The timing of drug administration is an important factor that must be carefully controlled in drug pharmacokinetic and pharmacodynamic studies and must be considered in planning dosing routes.

**Keywords** — valproic acid; anticonvulsant effect; drug concentration; circadian rhythm; biological rhythm; chronopharmacology; oral administration; rectal administration

Introduction

Valproic acid (VPA) is an anticonvulsant drug for the treatment of absence seizures and generalized tonic-clonic seizures. Oral, rectal and intravenous administration are available for the drug. So far, the oral route has been most widely used. The rectal administration is a suitable alternative in the case with impossibility of oral administration such as patients with severe vomiting, pre- and post-surgical patients, patients with ileus, patients on gastric suctioning, and patients in status epilepticus.1,2 The rectal route for treatment of refractory status epilepticus was first proposed by Vajda et al.3 Thus, the rectal route for VPA administration has an advantage according to the disease status of patients.

The pharmacotherapy based on the assumption that drug pharmacokinetic and pharmacodynamic processes are invariant with time has been mainly conducted to date. However, the timing of drug administration is of great interest for the pharmacotherapy of disease at present. It is also an important factor that must be considered in planning dosing routes and a controlled drug delivery system.4,5 Little information is available on the existence of circadian rhythm in drug pharmacokinetics and pharmacodynamics after rectal dose, although circadian changes in drug pharmacokinetics after oral dose have been demonstrated for a variety of drugs.6-8

Circadian stage-dependent changes of VPA anticonvulsant activity have been demonstrated in mice after oral administration. This rhythm is attributed to corresponding changes in the VPA absorption process.9 Namely, the time course of mean VPA concentrations in plasma and brain and mean electroshock seizure (ES)
threshold are higher in the middark phase than in the middark one, especially at 30 min (around $C_{\text{max}}$) after oral administration. The rate of drug absorption after oral administration is closely related to the gastric emptying time, since the intestines are a preferable site for VPA absorption. Food in the stomach delays the gastric emptying time. Therefore, circadian rhythm in the food intake of nocturnal animals, such as rodents, may play a major role in creating the rhythm in VPA absorption after oral administration. If so, the rectal administration of the drug may eliminate the effects of the amount of food remaining in the stomach on the VPA absorption, and consequently may eliminate the time-dependent changes of VPA absorption process. The observation and the possibility cited above prompted the present chronopharmacological study considering the route of drug administration.

This study was designed to investigate the influence of the dosing route on the chronopharmacological aspect of VPA in mice using the oral and rectal routes of administration of the drug.

Materials and Methods

Animals — Male ICR mice (7 weeks old) were used. Mice were housed 10 per cage in a standardized light-dark cycle (lights on at 0700, off at 1900) at a room temperature of 24 ± 1 °C and a humidity of 60 ± 10% with food and water available ad libitum.

Materials — Sodium valproate, a gift from Kyowa Hakko, Japan, was used at a dose of 400 mg/kg for oral and rectal routes. In the oral administration study, sodium valproate was dissolved in sterilized physiological saline to yield an appropriate concentration (800 mg/10 ml). The administered volume of sodium valproate solutions was 5 ml/kg. Oral administration was performed by insertion of a round-tip needle which was connected to a 0.5 ml syringe. In the rectal administration study, a suppository was prepared by mixing sodium valproate with a molten base of Witepsol H 15 (Mitsuba Trading, Japan). The suppository base without VPA was used for rectal dosing in the control group. A strong adhesive agent (Aron Alpha A, Sankyo, Japan) was used just after inserting a suppository into the rectum to prevent the evacuation of the suppository from the rectum. Diphenyl and other reagents were purchased from Wako (Japan) and were analytical grade and used without further purification.

Determination of ES Threshold — ES threshold was determined by a stimulator (E.C. stimulator model MK-800, Muromachi Company, Japan) increasing the direct current (0.1 mA) stepwise every prefixed period of time (0.2 s). The stimulus was delivered through copper corneal electrodes placed on the eyes. The ES threshold was defined as the amount of current in milliamperes delivered through corneal electrodes, which resulted in a detectable tonic forepaws flexion. This method can exactly evaluate the concentration-dependent change of VPA-induced anticonvulsant effect.

Determination of VPA Concentrations — The VPA concentrations in plasma and whole brain were determined by gas-liquid chromatography (GLC, Shimadzu GC-9A type gas chromatography; Shimadzu, Japan) with FID detector according to the procedure developed by Lobscher with slight modifications. One hundred µl 12 N hydrochloric acid and 100 µl chloroform containing diphenyl as an internal standard (30 µg/ml) were added to 100 µl plasma in a 400 µl tube. The whole brain tissue was homogenized in a 10 ml test tube using a mixture solution of 4 ml saline, 1 ml 12 N hydrochloric acid and 1 ml chloroform containing diphenyl as an internal standard (150 µg/ml). After the homogenates were shaken vigorously for 10 min, they were centrifuged 3000 rpm for 10 min, and the water layer was removed. Two µl of organic layer was applied into GLC with a 2 m × 3 mm glass column packed with 5% (FFAP) on 80/100 Gas-Chrom Q (Japan Chromato Kogyo, Japan). The column temperature was maintained at 170 °C, and the injection and detection temperatures were kept at 250 °C. Nitrogen, hydrogen and air flows were 30, 30 and 300 ml/min respectively. Quantitation was achieved by comparing peak area ratios (VPA to diphenyl). The coefficient of variation for assay error was less than 7% for GLC as exemplified by Lobscher.12
Procedure — To study the circadian rhythms of ES threshold and plasma VPA concentration, groups of 10 mice each were orally or rectally administered VPA, at the following schedule time: 0900, 1300, 1700, 2100, 0100 and 0500. A group of 10 mice each inserted with placebo base into the rectum served as controls. Mice were returned to their home cages (10 mice per cage) after VPA administration. Plasma and brain VPA concentrations reach the peak around 30 min after dosing in the midnight phase. ES threshold varies according to the pattern of both VPA concentrations and shows the peak around 30 min after dosing. Therefore, ES threshold was determined at 30 min after VPA administration. The blood samples were drawn by orbital sinus collection using micropipets (Drummond Scientific, U.S.A.) immediately after ES threshold determination. The plasma samples were obtained after centrifugation at 3000 rpm for 10 min (KN-70 Kubota, Japan) and then stored at −20°C until assayed. To study the time-dependent changes in brain VPA concentrations, groups of 10 mice each were orally or rectally administered VPA at the midnight during (1300) or at the middark (0100). Brain samples were obtained at 30 min after VPA administration and then stored at −20°C immediately after dissection and weight had been completed. To study the time course of plasma VPA concentrations, groups of 10 mice each were rectally administered VPA, at the midnight (1300) or at the middark (0100). The plasma VPA concentrations were determined at 5, 10, 15, 30, 45, 60, 75, 90, 105 and 120 min after rectal VPA administration.

Statistical Analysis — Statistical evaluation was performed by analysis of variance (ANOVA). Comparisons between treatments at light and at dark and comparisons between oral and rectal administrations were done by the Student’s t-test.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Time of administration</th>
<th>Route of administration</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oral (µg/ml)</td>
<td>Rectal (µg/ml)</td>
</tr>
<tr>
<td>Plasma VPA concentrations</td>
<td>0900</td>
<td>493.1 ± 80.7</td>
<td>576.0 ± 124.6</td>
</tr>
<tr>
<td></td>
<td>1300</td>
<td>590.2 ± 62.8</td>
<td>592.4 ± 195.0</td>
</tr>
<tr>
<td></td>
<td>1700</td>
<td>525.9 ± 97.2</td>
<td>590.0 ± 87.0</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>479.0 ± 53.1</td>
<td>582.0 ± 71.5</td>
</tr>
<tr>
<td></td>
<td>0100</td>
<td>409.1 ± 76.2</td>
<td>568.8 ± 141.8</td>
</tr>
<tr>
<td></td>
<td>0500</td>
<td>576.0 ± 80.3</td>
<td>576.0 ± 104.8</td>
</tr>
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</table>

ANOVAa) p<0.01 N.S.

a) Statistical evaluation for time-of-day effects of drug administration was performed by analysis of variance (ANOVA).
b) Comparisons between oral and rectal administrations were done by Student’s t-test. Each point represents the mean ± S.D. of 10 mice.
TABLE II. VPA Concentrations in the Brain at 30 min after Oral and Rectal Administration of VPA 400 mg/kg at the Midlight or at the Middark

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Time of administration</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1300 (Midlight)</td>
<td>0100 (Middark)</td>
</tr>
<tr>
<td>Oral</td>
<td>164.7 ± 49.3</td>
<td>99.5 ± 33.0</td>
</tr>
<tr>
<td>Rectal</td>
<td>175.3 ± 93.2</td>
<td>171.0 ± 60.9</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

All comparisons were done by Student’s \( t \)-test. Each point represents the mean ± S.D. of 10 mice.

**Results**

**Circadian Rhythm of ES Threshold**

There was a tendency to show the circadian rhythm in the ES threshold at 30 min after oral VPA administration \( (0.05 < p < 0.1) \) by ANOVA (Fig. 1). The mean ES threshold was significantly higher in mice orally administered with VPA at the 1300 (peak) as compared with that at 0100 (trough) \( (p<0.01) \). By contrast, there was no significant circadian rhythm in the ES threshold after rectal dosing. The mean ES threshold values after rectal dose were consistently higher than those after oral dose. Especially, a significant difference of ES threshold was demonstrated between rectal and oral routes, when VPA was administered at 0100 \( (p<0.01) \). There was no significant rhythm of ES threshold in the non-drugged state.

**Circadian Rhythm of Plasma VPA Concentrations**

In addition to the ES threshold, a significant circadian rhythm was also found in plasma VPA concentrations at 30 min after oral VPA administration \( (p<0.01) \) (Table I). The highest mean plasma VPA concentration was found in mice orally administered with VPA at 1300 and the lowest at 0100 \( (p<0.01) \). However, no significant circadian rhythm was found for plasma VPA concentration at 30 min after rectal dosing. These findings were consistent with the findings described above for the ES threshold. Also, the mean plasma VPA concentrations after rectal dose kept consistently higher levels in comparison to those after oral dose. When VPA was administered at 2100 or at 0100, there was a significant difference in plasma VPA concentrations between both routes \( (p<0.01) \), respectively.

**Circadian Change of Brain VPA Concentrations**

Brain VPA concentrations after oral dosing were significantly higher at the midlight than at the middark \( (p<0.01) \). However, no difference was found between two occasions after rectal dosing (Table II). These findings in brain VPA concentrations were closely related to those in the plasma VPA concentration and the ES threshold (Table I, II, Fig. 1). The mean brain VPA concentrations after rectal dosing showed higher levels compared to those after oral dose. When VPA was administered at 0100, a significant difference in brain VPA concentration was found between both routes \( (p<0.01) \).

**Time Course of Plasma VPA Concentrations after Rectal Dosing**

![Fig. 2. The Time Course of Plasma VPA Concentrations after a Rectal Administration of VPA (400 mg/kg) at 1300 or at 0100](image)

Each point represents the mean ± S.D. of 10 mice. •—•, administration of VPA at 1300; ○—○, administration of VPA at 0100.
Plasma VPA concentration reached a peak within 30 min after rectal administration and then gradually decreased (Fig. 2). Plasma VPA concentrations were not significantly different between administration at 1300 and 0100.

Discussion

Since the site of VPA action is in the central nervous system, the mechanism underlying the circadian rhythm of anticonvulsant effects has to be closely related with the rhythm in the brain VPA concentrations and/or the sensitivity of the brain to the drug. A significant circadian rhythm was demonstrated for ES threshold at 30 min after oral VPA administration, with the highest value at the midlight and the lowest at the middark. The rhythm varies according to that of VPA concentrations in plasma and brain showing the highest values at the midlight and the lowest at the middark. These findings were consistent with our previous report. The result also shows that the concentration-response relationship of VPA, namely the sensitivity to the drug, is not different between the midlight and the middark. Our present finding that there is no circadian rhythm of the ES threshold under the lack of rhythm in VPA kinetics in rectal dosing study coincides with our previous result in intraperitoneal dosing study. Therefore, the mechanism contributing to the circadian rhythm in the ES threshold is closely related to the corresponding changes in VPA kinetics.

In general, drug concentrations are influenced by the rates of four pharmacokinetic factors such as absorption, distribution, metabolism and excretion of drug. Plasma VPA concentrations at 30 min, around $C_{\text{max}}$, after oral dose are mainly reflected by the drug absorption rate from the upper gastrointestinal tract. This is supported by no time dependent changes of VPA kinetics in the present study with rectal route and the previous study with intraperitoneal route. Also, plasma VPA concentrations 30 min after rectal dosing are mainly attributed to the drug absorption rate from the rectal tract, since these are around $C_{\text{max}}$ in the time course of plasma VPA concentrations. Although plasma VPA concentrations at 30 min after dose indicate the drug absorption in both oral and rectal routes, only the oral route shows a significant circadian rhythm.

Drug absorption is often influenced by the amount of food in the stomach, since food induces changes in gastric secretion of hydrochloric acid, the rate of gastric emptying and intestinal transit time. The food and water intake of nocturnal animals such as rodents is confined mostly to the dark phase. The difference in the amount of food intake between the light and the dark phase may influence the rate of drug absorption from the upper gastrointestinal tract and may produce the circadian rhythm in VPA kinetics and in the ES threshold. By contrast, the rate of drug absorption from the rectal tract is not influenced by the amount of food or the gastric emptying time.

The VPA rectal suppository is useful for the patients who can not take the drug orally, since VPA solution for infusion has not been put on the market. In addition to the fact, the present results indicate that VPA rectal administration has an advantage in eliminating the time-dependent change in VPA absorption process after oral dosing without losing much anticonvulsant effect. However, the present finding may differ from that found in clinical practice. Since rodents such as mice and rats are nocturnal and humans are diurnal, generally there are some kinds of differences in physiological function and pharmacologic action expected between humans and animals. VPA CL in rodents is about 70 fold higher than that in human. Therefore, dosage differs largely between human and animal studies. VPA shows a different metabolism and enterohepatic recirculation in relation to the dose used. It can be expressed more exactly that VPA has different kinetics depending on its concentration. Also there is a different rhythm of VPA kinetics depending on the concentration. No circadian rhythm is shown for $V_d$ and CL at a high VPA concentration over 100 $\mu$g/mL. On the other hand, significant circadian rhythm is demonstrated for $V_d$ and CL at a low VPA concentration less than 100 $\mu$g/mL. However, no information is available on the concentration-dependent kinetics in VPA absorption and the different rhythm of VPA absorption according
to the concentration. Future research is required to determine whether the findings based on animal experiment are extrapolated to human.

To summarize, dosing time significantly influences the extent of VPA absorption and anticonvulsant effect after oral dose. Thus the timing of drug administration is an important factor that must be carefully controlled in drug pharmacokinetic and pharmacodynamic studies. It is also an additional factor that must be considered in planning dosing routes and in designing drug formulations.

References