±45 nmol·L$^{-1}$增加到621±121 nmol·L$^{-1}$. **结论**: MK-447的血小板变形与其[$Ca^{2+}$]$_i$释放有关. MK-447增强凝血酶的血小板聚集和ATP释放, MK-447的这一作用可能于[$Ca^{2+}$]$_i$的协同作用有关.

**关键词** 血小板聚集; 二磷酸腺苷; 钙; 凝血酶; MK-447

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# Chronopharmacokinetics of valproic acid following constant-rate administration in mice and influence of feeding schedule

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**AIM**: To study the circadian rhythm in pharmacokinetics of valproic acid (VA) and influence of feeding schedule on the rhythm. **METHODS**: Sodium valproate was administered by osmotic minipump technique (1.062 mg·h$^{-1}$) and iv (50 mg·kg$^{-1}$) to ICR mice fed under *ad lib* or time-restricted schedules to determine the time-dependent changes of VA kinetics. **RESULTS**: Plasma VA concentration and clearance at steady-state showed circadian rhythms ($P<0.01$). Time-restricted feeding influenced the rhythm of VA kinetics, acrophases of rhythms shifted approximately 12 h. **CONCLUSION**: Timing of dosing is important for VA kinetics and feeding schedule is one of synchronizers in VA kinetics.

**KEY WORDS** valproic acid, pharmacokinetics, circadian rhythm, drug administration schedule

Valproic acid (VA) is an antiepileptic drug. The toxicity, anticonvulsant actions and kinetics of VA showed circadian rhythm changes in rodents. The circadian rhythm of plasma VA concentrations corresponded well to that of VA anticonvulsant actions and related to the feeding condition[1,2]. The present work was to study the circadian rhythmicity of plasma VA concentration in mice following constant-rate VA administration using osmotic minipumps and to identify the role of feeding schedule on the circadian rhythm of kinetics of VA.

## MATERIALS AND METHODS

ICR mice, ♂, 6-wk old (30.2±*s* 2.8 g), were housed 10 per cage from 4-wk old in a standardized light-dark cycle of light on 7:00—19:00, at a room temperature of 24±1 ℃ and a humidity of 60±10 % with food (Oriental Yeast Co, Tokyo, Japan) and water *ad lib* or under a time-restricted feeding schedule (feeding time: 9:00—17:00).

In the study observing the circadian rhythm in plasma VA concentration at the steady-state, 2 groups of 10 mice fed under *ad lib* or time-restricted schedules were anesthetized with ether. A small incision was made in the bake of mice and 2 osmotic minipumps (Model 2001 with 25 mm in length, 7 mm in diameter, Alzet Corp, USA) were implanted subcutaneously into the pockets. The concentration of VA solution used filling one pump was 600 g·L$^{-1}$ of sodium valproate (Valerin, Dainipon Pharmaceutical Co, Japan).

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Each pump released VA into the subcutaneous area in a continuous and constant rate (1.02 $\mu L \cdot h^{-1}$). Since VA/sodium valproate ratio is 0.8677, VA release rate is 600 $g \cdot L^{-1} \times 0.8677 \times 1.02\ \mu L \cdot h^{-1} \times 2 = 1062\ \mu g \cdot h^{-1}$. VA elimination half-life in rodents is shorter than 1 h[3], 7 h after the pump implantation is the time enough to attain a steady-state plasma concentration. Multiple samples (60 $\mu L$ for each sample) were drawn from the experimental and control mice by orbital sinus collection using micropipettes before and at 24, 28, 32, 36, 40, 44 h after the pump implantation. Plasma VA clearance (*Cl*) at steady-state were calculated as following:

$$Cl\ (L \cdot kg^{-1} \cdot h^{-1}) = 1.062\ (mg \cdot h^{-1}) / Wt\ (kg) \times Cp\ (mg \cdot L^{-1})$$

Wt: body weight, Cp: plasma VA concentration.

Forty mice fed under *ad lib* schedule ($n=20$) and time-restricted schedule ($n=20$) were used to study the time course of plasma VA concentration. Ten mice per group were injected sodium valproate (50 $mg \cdot kg^{-1}$, iv) at 17:00 and 5:00. Plasma VA concentration was determined at 15, 30, 45, 60, 90 min after iv VA. Pharmacokinetic parameters were calculated following one-compartment model[4]. Plasma VA concentration was analysed by homogeneous enzyme immunoassay technique (EMIT, Syva Co, USA). The coefficient of variation for assay error is <10 %[5].

ANOVA and *t*-test were used for the statistical analysis. The cosinor method was used to calculate mesor, amplitude and acrophase of circadian-rhythmometry.

## RESULTS

**Circadian rhythms of VA plasma concentration and Cl** There was circadian variation in plasma VA concentration at steady state after minipump implanted into *ad lib* mice for 24 h ($P<0.01$, ANOVA). The mean plasma VA concentrations were higher during the light phase than the dark phase with peak at 17:00 (55.1 ± 12.3 $mg \cdot L^{-1}$) and trough at 5:00 (25.6 ± 6.0 $mg \cdot L^{-1}$). There was also a circadian variation in *Cl* ($P<0.01$), which showed a mirror image with plasma VA concentration with the highest value at 5:00 (1.316 ± 0.366 $L \cdot kg^{-1} \cdot h^{-1}$) and the lowest one at 17:00 (0.597 ± 0.131 $L \cdot kg \cdot h^{-1}$). The best-fit single cosine curves of plasma VA concentration and *Cl* were $\hat{Y}_t = 35.8 + 13.9 \cos(15° \cdot h^{-1} \cdot t_i - 236°)$ and $\hat{Y}_t = 1.055 + 0.414 \cos(15° \cdot h^{-1} \cdot t_i - 47°)$, respectively.

**Influence of time-restricted feeding on circadian rhythms of plasma VA concentration and Cl** Time-restricted feeding schedule had a marked influence on circadian rhythm of VA kinetics. Plasma VA concentration was higher during the dark phase than that of the light phase ($P<0.01$) with peak at 5:00 (55.3 ± 18.4 $mg \cdot L^{-1}$) and trough at 17:00 (25.1 ± 4.3 $mg \cdot L^{-1}$). The rhythm of *Cl* was also shifted with peak at 17:00 (1.394 ± 0.263 $L \cdot kg^{-1} \cdot h^{-1}$) and trough at 5:00 (0.712 ± 0.266 $L \cdot kg^{-1} \cdot h^{-1}$). The best-fit single cosine curves of plasma VA concentration and *Cl* were $\hat{Y}_t = 38.0 \pm 15.1 \cos(15° \cdot h^{-1} \cdot t_i - 45°)$ and $\hat{Y}_t = 1.039 \pm 0.367 \cos(15° \cdot h^{-1} \cdot t_i - 220°)$, respectively. Acrophase of the rhythms under time-restricted feeding schedule was shifted about 12 h in comparison to that under *ad lib* feeding schedule. There were no obvious changes on mesor or amplitude between the 2 schedules (Fig 1).

**Circadian variation of VA kinetics** Mean plasma VA concentration was higher in *ad lib* feeding mice, iv VA 50 $mg \cdot kg^{-1}$ at 17:00 than that at 5:00, at 15 min (97.7 ± 8.7 and 77.5 ± 17.6 $mg \cdot L^{-1}$, $P<0.01$), 30 min (48.7 ± 10.5 and 36.9 ± 8.2 $mg \cdot L^{-1}$, $P<0.05$), and 45 min (26.6 ± 5.8 and 21.4 ± 4.9 $mg \cdot L^{-1}$, $P<0.05$) after injection. The volume of distribution ($V_d$) was larger ($P<0.05$), *Cl* was higher ($P<0.01$) and area under the curve (AUC) was smaller ($P<0.01$) in mice injected with VA at 5:00 than those at 17:00.

**Influence of time-restricted feeding schedule on circadian variation of VA kinetics** Under time-restricted feeding schedule, char-

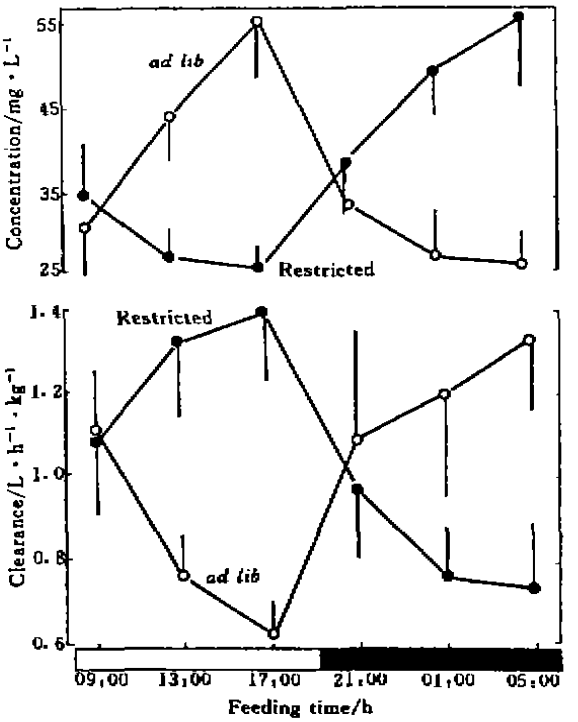


**Fig 1. Plasma concentration and clearance of valproic acid following constant-rate administration. $n=10$ mice. $\bar{x}\pm s$.**

acteristic of circadian variation in VA kinetics was influenced. Plasma VA concentration was higher in mice injected with VA at 5:00 than that at 17:00, at 15 min (104.1±11.1 and 90.7±10.5 mg·L$^{-1}$, $P<0.01$), 30 min (55.2±10.6 and 46.7±4.5 mg·L$^{-1}$, $P<0.05$), and 45 min (31.9±7.5 and 24.0±5.9 mg·L$^{-1}$, $P<0.05$). $V_d$ was smaller ($P<0.05$), Cl was lower ($P<0.01$) and AUC was larger ($P<0.01$) in mice injected with VA at 5:00 than those at 17:00 (Fig 2, Tab 1).

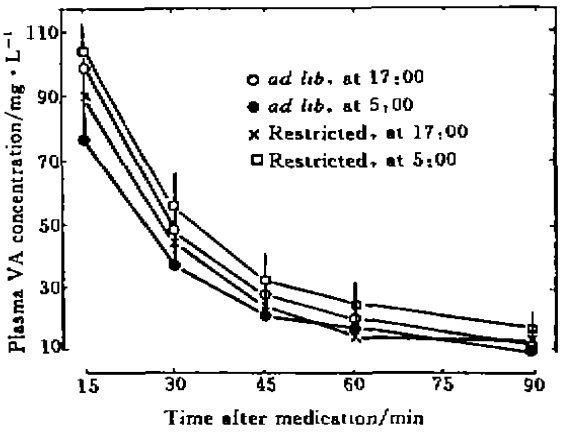


**Fig 2. Plasma concentration after iv VA 50 mg·kg$^{-1}$. $n=10$ mice. $\bar{x}\pm s$.**

## DISCUSSION

The present study shows that the constant-rate administration of VA does not produce constant plasma VA concentration, which was higher in the light phase than that in the dark phase. A circadian rhythm was found for *Cl*, showing a mirror image with that of plasma VA concentration. It suggested that the circadian rhythm of *Cl* might be one of the mechanisms resulting in the circadian change in plasma VA concentration. The rhythms of body temperature (BT) and blood

**Tab 1. Influence of dosing time and feeding schedule on pharmacokinetic parameters of valproic acid in mice. $n=10$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* the groups iv at 17:00.**

| | *Ad lib* feeding | | Time-restricted feeding | |
|---|---|---|---|---|
| | 17:00 | 5:00 | 17:00 | 5:00 |
| $V_d$(L·kg$^{-1}$) | 0.34±0.03 | 0.49±0.20$^b$ | 0.38±0.05 | 0.33±0.05$^b$ |
| $K_e$(h$^{-1}$) | 2.05±0.29 | 1.98±0.69 | 2.08±0.29 | 1.95±0.38 |
| $T_{1/2}$(h) | 0.35±0.06 | 0.39±0.15 | 0.34±0.05 | 0.37±0.09$^c$ |
| *Cl* (L·kg$^{-1}$·h$^{-1}$) | 0.70±0.10 | 0.86±0.06$^c$ | 0.74±0.07 | 0.62±0.07$^c$ |
| AUC (mg·L$^{-1}$·h$^{-1}$) | 73.5±11.2 | 58.9±3.6$^c$ | 68.0±6.0 | 81.0±8.7 |

flow rate (BFR) of mice might influence VA release rate and its absorption (Alzet osmotic pump technical information manual). But these rhythms were in opposion to those of plasma VA concentration, in which the values were lower during the dark phase although the circadian rhythms in BT and BFR peaked during this phase[6,7].

The results demonstrated that VA kinetic parameters, *Cl* and $V_d$ showed dosing time-dependent change, suggesting that the circadian changes in *Cl* and $V_d$ might contribute to that of VA chronopharmacokinetics. VA was cleared by liver[8], *Cl* of VA is restricted by heptic BFR, which is higher during activity period (dark phase) than rest period (light phase) in rodents[7]. Our finding on the circadian rhythm of *Cl* corresponded nicely to the rhythm of hepatic BFR. Therefore, higher liver BFR might contribute to the increase of *Cl* during the activity period of mice.

Manipulation of feeding schedule definitely modifies the rhythm of VA kinetics and the circadian aspects of plasma VA concentration, *Cl* and $V_d$ were reversed under time-restricted feeding schedule. Since the food and water intake of rodents was confined to the active period[9,10], restricted food availability substantialy modified circadian pattern of an animal's behavioral and physiological activities, including enzyme activity, blood circulation and urinary excretion[11,12]. The circadian rhythms of gentamicin and methotrexate kinetics were modified by feeding conditions, showing that feeding schedule was one of the most important factors influencing circadian rhythms in pharmacokinetics of drugs[13,14].

Since the kinetics of VA showed rhythmic changes and was influenced by feeding conditions, the choice of the most appropriate timing of drug administration in relation to the feeding schedule might be helpful for rational usage of the drug.

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## 丙戊酸恒速给药在小鼠体内时间药物动力学及进食条件的影响

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**目的**：研究丙戊酸(VA)药物动力学昼夜节律变化及进食条件对节律的影响. **方法**：对自由进食及限定进食的 ICR 小鼠分别以渗透压微泵技术(1.062 mg·h$^{-1}$)及 iv (50 mg·kg$^{-1}$)给予丙戊酸钠，并测定 VA 动力学的时间依赖性变化. **结果**：血浆 VA 浓度及清除率在稳态时呈昼夜节律性变化($P$<0.01)，限定进食时间影响 VA 动力学的节律，使峰值位相移动约12 h. **结论**：用药时间是影响 VA 药动学的重要因素，进食条件是 VA 药动学节律的同步因子之一.

**关键词** 丙戊酸；药物动力学；昼夜节律；用药计划表

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# Absorption of indometacin from nasal cavity in rats

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**AIM**: To investigate if identical bioavailability, rapid $T_{max}$, and $C_{max}$ of indometacin (Ind) could be achieved when Ind is administered in rats via intranasal (ina) route. **METHODS**: The pharmacokinetics of Ind solution at a dosage of 3 mg·kg$^{-1}$ was studied after iv, ina, and *po* in rats using HPLC. **RESULTS**: It showed that the time to peak ($T_{max}$) of ina Ind 3 mg·kg$^{-1}$ solution was 0.08 h, approached that after iv route the peak concentration ($C_{max}$) following ina was 20.0 mg·L$^{-1}$, 2.4 times higher than *po* dosing. **CONCLUSION**: It demonstrated that the ina administration of Ind was superior to *po* in rats, and that Ind absorption through nasal mucosa was a reasonable approach at lower doses.

**KEY WORDS** indomethacin; high pressure liquid chromatography; intranasal administration; pharmacokinetics

Indometacin (indomethacin, Ind) is an anti-inflammatory and analgesic-antipyretic drug in experiments, but produces erosions and ulcers in the gastrointestinal tracts[1,2]. Our laboratory showed that index of Ind-induced ulcer was highest in *po*, then iv, and sc routes. If there is an alternative route of administration through which Ind could be at a lower dose to avoid gastric irritation[3], while at the same time is enough to produce desired pharmacological effects[4]? The present study is to investigate if identical bioavailability, rapid $T_{max}$, and $C_{max}$ of Ind could be achieved when Ind is administered in rats via intranasal (ina) route.

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