Valproic Acid Elimination Rate and Urinary Excretion of Its Glucuronide Conjugate in Patients with Epilepsy

Hisahiro YOSHIDA, a Kiyoshi HIROZANE, a Hiroyo KIMOTO, a Takasi HAYASHI, b Tatsuo AKIYAMA, c Hirokazu KATAYAMA, d Miki WATANABE, d Hironori YOSHITOMI, d and Akira KAMIYA d,a

Department of Pharmacy, Yamaguchi University Hospital,a Department of Pediatrics, b Department of Neurosurgery, c Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube 755-8505, Japan and Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, d Gakuen-cho, Fukuyama 729-0292, Japan.

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We previously encountered a patient with epilepsy who exhibited rapid elimination of sustained-release valproic acid (VPA) administered at the dose of 2.8 g/d as a sodium salt. The purpose of this study was to clarify the relationship between the VPA elimination rate and the proportion of the dose excreted in urine as its glucuronide conjugate (VPA-G) in epileptic patients. Twenty-four-hour urine was collected from four epileptic patients who had taken VPA orally (age: 16—39y, weight: 50—63 kg, dose: 1.0—2.8 g/d). VPA and its metabolites were detected by gas chromatography-mass spectrometry. The amounts of VPA, VPA-G, 3-keto VPA, and 3-OH VPA excreted in the 24-h urine were 1.8—13.2, 178—2158, 125—320, and 8.6—18.7 mg (converted into VPA), respectively, and 0.2—0.5, 20.5—88.7, 5.8—18.7, and 0.6—1.0% of the dose administered, respectively. The dose of VPA correlated well with the proportion of the dose excreted in urine as VPA-G in each patient, and the patients administered a high dose excreted a large amount of VPA-G in the urine. Therefore, the differences in the VPA-G production rate may be one of the major variable factors affecting the elimination of administered VPA. We also present a dynamic model of VPA in the kidney which may explain the VPA elimination phenomena in humans on the basis of the data obtained here regarding the concentrations of VPA and its metabolites in plasma and their urinary excretion levels.

Key words valproic acid; glucuronide; metabolism; kidney; epileptic patient; urinary excretion

Valproic acid (VPA) is a branched chain fatty acid used widely as an antiepileptic drug. Its molecular weight is 144.2. VPA seems to be metabolized by various pathways, and its metabolites are mainly excreted in the urine and bile.1—4 VPA is conjugated with glucuronic acid by uridine diphosphate-glucuronosyl-transferase (UGT), and metabolized to 3-OH VPA and 3-keto VPA via β-oxidation or α2-oxidation, to 5-OH VPA and 2-propylglutaric acid via ω-oxidation, and to 4-OH VPA and 4-keto VPA by ω1-oxidation. It is well known that the main metabolite in plasma is 3-keto VPA, whereas that in urine is VPA glucuronide (VPA-G). The kinetics of VPA in the body are complicated and differ markedly among individual patients.5—7 Accordingly, the plasma concentrations of VPA in each patient should be monitored in order to optimize pharmacotherapy.

We previously encountered a patient with epilepsy who exhibited a rapid elimination of sustained-release VPA administered at a dose of 2.8 g/d.8—10 In the present study, we examined the relationship between the VPA elimination rate and the proportion of the dose excreted in urine as VPA-G in epileptic patients. We also present a dynamic model of VPA in the kidney based on the data obtained here regarding the concentrations of VPA and its metabolites in the plasma and their urinary excretion levels.

MATERIALS AND METHODS

Materials Sodium valproate and undecylenic acid were purchased from Wako Pure Chemicals Ind., Ltd. 3-OH valproic acid (3-OH VPA) and 3-keto valproic acid (3-keto VPA) were kindly supplied by Kanabe Co. and Dr. Muro (Maji Rosai Hospital), respectively. All other chemicals and solvents used were of analytical reagent grade.

Subjects and Methods Four epileptic patients (age: 16—39y, weight: 50—63 kg), who had taken VPA chronically (dose: 1.0—2.8 g/d) with other antiepileptic drugs (Table 1), were included in this study after informed oral consent. The collection of 24-h urine was started at 6 a.m. and finished the next day at 6 a.m. The part of the urine used to measure volume was stored at −40°C until use. Plasma was obtained by centrifugation and stored at −40°C until use.

Assays The concentrations of VPA, VPA-G, 3-keto VPA, and 3-OH VPA in 24-h urine and blood were detected using gas chromatography—mass spectrometry (GC-MS) (QP-5000, Shimadzu, Kyoto, Japan), according to the method of Tsutsuhara et al.11 In brief, after VPA, 3-keto VPA, and 3-OH VPA in the specimens were extracted in ethylacetate at pH 5.0, the organic solvent was concentrated under a stream of N2 gas. Their function groups were then protected with N-methyl-N-trimethylsilyl trifluoracetamide. The mixture was injected into the GC-MS system. VPA-G in the specimens was hydrolyzed to VPA with β-glucuronidase (type X-A, Sigma, St. Louis, MO, USA).12 Namely, 10μl of patient urine was mixed with 100μl of phosphate buffered saline (PBS, pH 6.8) containing 1000 units of β-glucuronidase, and those were incubated at 37°C for 30 min. Then, the VPA generated was measured as VPA-G by the GC-MS method mentioned above.

Estimation of Pharmacokinetic Parameters The pharmacokinetic (PK) parameters of VPA and phenytoin in each patient were estimated by the Bayesian forecasting method using Horii’s population mean parameters13,14 and YUHR-tdm analysis software.

Prediction of Mean Plasma Concentrations Prediction of the mean plasma concentrations (Cpme) of VPA and its metabolites in each patient was performed on the basis of

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those amounts excreted in 24-h urine ($E_{ur}$). These concentrations were calculated on the hypothesis that in each patient the glomerular filtration rate (GFR) was equal to the creatinine clearance ($C_{cr}$). The protein binding levels ($PB$) of VPA and its metabolites were 85 and 0%, respectively, and the excretion route for each compound was only by glomerular filtration (the reabsorption and secretion routes did not contribute). The $C_{cr}$ was calculated using the serum creatinine concentration in each patient according to Jelliffe’s formula.

Thus, $C_{cr}$ was calculated as follows: $C_{cr} = (\text{mg/ml}) \times (\text{ml/min} \cdot (1 - PB/100) \cdot 60 \times \text{[h]} \cdot 24 \times \text{[h]}$. 

### Amount of VPA Filtrated by Glomeruli

The amount of VPA filtrated by renal glomeruli ($A_{G}$) was calculated as follows: $A_{G} = C_{cr} \times (\text{mg/ml}) \times (1 - PB/100) \times C_{cr} \times (\text{ml/min}) \times 60 \times \text{[h]} \times 24 \times \text{[h]}$. It was assumed that the $PB$ of VPA in each patient was 85%, and that the GFR was equal to $C_{cr}$.

### RESULTS

#### Plasma Concentrations of VPA and Its Metabolites

As shown in Table 1, the plasma concentrations of VPA, 3-OH VPA, 3-keto VPA, and VPA-G in the patients ranged from 61.3—95.2, 0.07—0.3, 3.2—16.6 μg/ml, or were nondetectable, respectively. VPA plasma concentrations in each patient were well within the therapeutic range (50—100 μg/ml), except for patient #4 who exhibited periodic increases above the effective range for a few hours after VPA administration.

The PK parameters of VPA in each patient were calculated and are shown in Table 2. The distribution volume of VPA was similar for each patient, though the absorption rate constant and the elimination rate constant were markedly different among the patients. The elimination rate constants in the patients were correlated with their doses. The PK parameters of phenytoin in three patients who also took phenytoin were analyzed. These values were similar for all patients and were nearly equal to those values obtained from general patients. The PK parameters of other concomitant drugs in the patients were similar to those obtained from general patients.

### Amounts of VPA and Its Metabolites in Urine

The levels of VPA and its major metabolites were measured in the 24-h urine of each patient. The excretion amount of VPA was low but ranged rather widely (from 1.8 to 13.2 mg) among the patients (Table 3). The levels of 3-OH VPA and 3-keto VPA in the 24-h urine ranged from 8.6 to 18.7 mg and from 125 to 320 mg, respectively, and these fluctuation ranges were narrower than that of VPA. In contrast, the excretion amount of VPA-G varied quite widely among the patients, from 178 to 2158 mg. The urinary excretion amount of VPA-G in each epileptic patient differed markedly from that of other metabolites. The VPA dose for each patient correlated with their urinary excretion amounts of VPA-G (Fig. 1A).

### Relation between the VPA Clearance and the Urinary Excretion of Its Metabolites

The relationship between the urinary excretion amount, or the proportion of VPA and its metabolites to the VPA clearance, was examined. As shown in Fig. 1B and C, the VPA clearance correlated well with the urinary excretion amount of VPA-G and with the proportion.
of the dose excreted in urine as VPA-G, but not with the amounts of 3-OH and 3-keto VPA.

**Prediction of the Concentration of VPA and Its Metabolites in Plasma** The mean plasma concentrations of VPA and its metabolites were predicted from the excretion amount in the 24-h urine of each epileptic patient. As shown in Table 4, the predicted mean plasma concentrations of VPA, 3-OH VPA, 3-keto VPA, and VPA-G were 0.03—0.56, 0.05—0.08, 0.7—1.4, and 0.37—9.8μg/ml, respectively. The 3-OH VPA concentrations in plasma were similar to the values predicted from urinary excretion amounts, and the 3-keto VPA concentrations in plasma were about five to twelve times higher than that predicted from excretion amounts. The VPA values predicted from urinary excretion amounts were markedly different from actual plasma concentrations. Moreover, VPA-G could not be detected in the patients' plasma, but the presence of VPA-G was predicted in the patients' plasma according to the urinary excretion amount.

**Movement of VPA in Kidney** The amounts of VPA filtered by renal glomeruli and reabsorbed from renal tubules in each patient were calculated. As shown in Table 5, the amount of VPA filtered at the glomeruli in each patient was 1411 to 2876 mg/d. The urinary excretion amount of VPA in each patient was very small and its percentage was lower than 1% of the amount of VPA filtered at the renal glomeruli. More than 99% of VPA filtered at the renal glomeruli was reabsorbed from renal tubules into the epithelial cells. In patient #3, the urinary excretion amount of VPA-G was similar to the amount of VPA reabsorbed from renal tubules, while in other patients the excretion amount of VPA-G was lower than the amount of VPA reabsorbed.

### DISCUSSION

In this paper, we measured the VPA plasma concentrations of 4 epileptic patients taking VPA for long periods. The population PK parameters of each patient were calculated based on these values by the Bayesian method. A good correlation was recognized between VPA dose and the urinary excretion amount of VPA-G in the patients, between VPA clearance and the urinary excretion amount of VPA-G, and between VPA clearance and the proportion of the dose excreted in urine as VPA-G. These results indicate that one of the major variable factors affecting the dose of VPA is the proportion of the dose excreted in urine as VPA-G (% VPA-G).

An equation was obtained from the correlation between VPA clearance (Cl) and % VPA-G (Fig. 1C).

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Cl = 0.017 \times (\% \text{ VPA-G}) + 0.309
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Generally, the medicinal daily dose \( (D_d) \) can be predicted by the following equation.
When Eq. 1 was substituted for Eq. 2, the following equation was obtained.

$$D_p = C_{pe} \times \left( 0.017 \times \text{(% VPA-G)} + 0.399 \right) \times 24$$  \hspace{1cm} (3)

This equation indicates that we can predict the optimal VPA dose to each patient from the proportion of the dose excreted in urine as VPA-G.

When the drug concentration in blood is remarkably lower than the predicted value calculated for the dose, we usually consider this to be due to decreasing patient compliance. However, the same phenomena can be observed when the drug metabolic functions are remarkably accelerated in the patient. In the case of psychiatric disorder patients, to construct a good fiduciary relation between the medical staff and the patient is especially important for pharmacotherapy and various medical treatments. Repeated blood sampling from these patients is not easy, since they are oversensitive compared to normal subjects. On the other hand, urine is suitable as a biological specimen for analyzing drug concentrations, and its collection can be easily carried out without upsetting patients. In this paper we clarified that the VPA dosage required for each patient could be easily predicted by measuring the proportion of the dose excreted in urine as VPA-G in each patient. These results indicate that it is possible to avoid unnecessary trouble between patients and medical staff regarding taking (or nontaking) medicine and to construct a good fiduciary relationship between them.

Large differences in VPA absorption rate constants ($K_1$) among patients may be due to differences in the pharmaceutical preparations which were administered. Depakene is a conventional tablet, while depakene R and selenica R are sustained-release tablets. The absorption rate of drugs in the intestine generally correlates well with the soluble velocity of preparations. The larger $K_1$ value in patient #4 is probably due to the administration of conventional tablets of VPA.

The plasma concentrations of VPA might exceed the upper limit of the therapeutic range (50—100μg/ml) in patient #4. To maintain the plasma concentration of medicines in the therapeutic range is advisable to avoid adverse effects. We advised the doctor of patient #4 that the preparation should be changed to a sustained-release type in order to maintain the VPA blood concentration within the therapeutic range. However, as the patient’s epilepsy had been well controlled without showing adverse effects, the doctor wanted to continue the present pharmacotherapy for this patient.

The liver is considered the main organ which metabolizes VPA to VPA-G. However, if VPA-G is generated in organs other than the kidney, VPA-G should be detected in the patients’ blood; VPA-G generated in organs other than the kidney would first be removed from the organs into the blood and secondly excreted in urine by the kidney. Namely, if VPA-G was formed in organs other than the kidney in patient #3, the mean blood concentration of VPA-G would be predicted to be 9.8μg/ml based on the urinary excretion amount of VPA-G. However, we could not detect VPA-G in the patients’ blood near the maximum blood concentration level of VPA. The VPA-G amount excreted in the urine of each patient was the same as or less than the VPA amount filtered in the glomeruli. These results suggest that the kidney is a major production organ for VPA-G. We assumed that the PB of VPA in each patient was 85%. After that, we measured the PB of VPA in our 4 patients, and obtained values of 84 to 86%. These results indicate that our assumed conditions reflect the actual situation.

A dynamic model of VPA in the kidneys is presented in Fig. 2. In this model, most VPA administered orally is absorbed from the gastrointestinal tract and removed into systemic circulation. Some of the VPA is infused in the kidneys, and unbound VPA is filtered by glomeruli. Most of the VPA (>99%) removed into the renal tubules is removed in the tubular epithelial cells by simple diffusion, as VPA is a lipophilic acidic drug. Most of the VPA removed into the epithelial cells seems to be quickly carried out in the capillaries by an organic anion transport system in the basolateral membrane (BLM) such as OAT-K2. It is known that UGT and uridine diphosphate-glucuronic acid (UDP-GA) are located in tubular epithelial cells. The production rates of VPA-G depend on the activity of UGT and the amount of UDP-GA. The VPA-G formed is quickly excreted into the renal tubules by certain transport systems such as MOAT (multispecific organic anion transporter). In patient #3, the urinary excretion amounts and proportions of VPA-G were markedly different among the patients. The reasons may be as follows: (1) the organic anion transport system in BLM was inhibited by VPA-G produced in the epithelial cells, (2) the organic anion transport system is deficient, (3) UGT in the epithelial cells has high activity and is rapidly metabolized from VPA to VPA-G, and/or (4) abundant UDP-GA exists in the epithelial cells and accelerates UGT to metabolize VPA to VPA-G.

Further studies to investigate the accuracy of the model presented in this paper are underway.

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