Urinary Metabolites of Valproic Acid in Epileptic Patients

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Received September 26, 1997; accepted December 15, 1997

The urinary excretion of valproic acid (VPA) and its metabolites (3-keto VPA, 3-OH VPA, and VPA-glucuronide) in 6 epileptic patients was studied using gas chromatography-mass spectrometry. The amount of VPA and 3-OH VPA excreted in the urine was low (0.1–0.5% of the dose of VPA and 0.6–1.5% of the dose of 3-OH administered). The amount of 3-keto VPA and glucuronide (VPA-Glu) excreted was marked (5.8–26.2% and 13.1–88.7% of the dose of VPA administered, respectively). The urinary excretion of VPA and its metabolites by patients who have taken a normal amount of a VPA preparation was almost the same as that of healthy volunteers. Two epileptic patients who took a large amount of the VPA preparation showed a high excretion of VPA-Glu without an increase in their plasma VPA-Glu.

Key words valproic acid; epileptic patients; urinary excretion; glucuronide; GC-MS

Valproic acid (dipropylacetic acid, 2-propylvaleric acid, VPA) is a branched-chain fatty acid and is commonly used as an antiepileptic drug. It is effective against many types of seizures; specifically, it is the only available antiepileptic drug that is effective against absence and other types of generalized seizure. VPA inhibits sustained repetitive firing induced by depolarization of the mouse cortical or spinal cord neurons.¹⁾ Although VPA has no effect on responses to γ -aminobutyric acid (GABA), the amount of GABA in the brain is increased by VPA administration in animals. It has been difficult to correlate the increase in GABA levels to the activity of VPA.²⁾

VPA is completely absorbed after oral administration of a commercial tablet containing sodium valproate.^{3,4)} VPA binds to plasma protein (mainly albumin) at 90–95% efficiency in the therapeutic range.^{5,6)} VPA can be used alone or as a supplementary medication. The therapeutic range of VPA in plasma is 50–100 $\mu\text{g}/\text{ml}$.⁷⁾ VPA shows a large inter-subject variance in plasma concentration, because of age^{8,9)} drug interaction,^{10,11)} or a change in protein binding.^{12,13)}

VPA is metabolized by β -oxidation, glucuronidation, etc., and the metabolites are excreted into the urine (Fig. 1). The percentage of VPA and its metabolites excreted in the urine

might be related to the intersubject variation of VPA plasma concentration. Although there have been studies on urinary VPA and its metabolites performed on healthy volunteers,^{14–17)} there have been few studies on epileptic patients. Among the various methods for the determination of VPA in biological fluids, gas chromatography-mass spectrometry (GC-MS) is the most sensitive for the simultaneous determination of VPA and its metabolites.^{18,19)}

In this study, urinary metabolites, including the VPA of 6 epileptic patients who are undergoing chronic treatment with VPA, were studied by GC-MS.

MATERIALS AND METHODS

Materials Sodium valproate and undecylenic acid were purchased from Wako Pure Chemicals Co., Ltd. 3-OH valproic acid (3-OH VPA) and 3-keto valproic acid (3-keto VPA) were kindly supplied by Kanebo Pharm., Ltd. and Dr. Muro (Moji Rosai Hospital).¹⁸⁾ All other chemicals and solvents used were of analytical reagent grade.

Subjects and Methods Six epileptic patients, who take VPA chronically with or without other antiepileptic drugs, were included in this study after informed oral consent

Fig. 1. Metabolic Pathways of Valproic Acid

1: β -oxidation, 2: (ω -2)-oxidation, 3: (ω -1)-oxidation, 4: ω -oxidation, 5: δ -oxidation, 6: γ -oxidation, 7: conjugation.

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Table 1. Characteristics of Epileptic Patients Undergoing Chronic Valproic Acid Treatment

Patient	Y.T.	K.S.	F.T.	N.T.	I.T.	N.S.
Sex	M	F	M	M	M	M
Age (yrs)	55	39	67	39	20	16
Weight (kg)	76	63	50	54	58	57
Height (m)	1.68	1.62	1.57	1.65	1.63	1.74
Preparations	Selenica R	Selenica R	Hyserenin	Selenica R	Depakene R	Depakene
Dose ^{a)} (g/d)	0.8	1.0	0.8	1.2	2.8	2.8
Concomitant drugs ^{b)} (dose; g/d)	nothing	PB (0.12)	nothintg	DPH (0.3) DP (0.004) PRM (0.45)	DPH (0.37) PRM (0.35)	DPH (0.75) ESM (0.5)

a) as VPANA. b) Concomitant drugs that effect the pharmacokinetics of VPA are listed. DPH, phenytoin; PRM, primidone; ESM, ethosuximide; PB, phenobarbital; DP, diazepam.

Fig. 2. Plasma Concentration of VPA and Its Metabolites in Epileptic Patients

○, VPA; ●, 3-OH; △, 3-keto; □, VPA-Glu. Blood sampling time was the passage of time after the last administration: 2 h, K.S.; 12 h, N.T.; 4 h, I.T.; 12 h, N.S. a) not determined.

(Table 1). Plasma was obtained by centrifugation and stored at -40°C until being assayed. A part of the urine, that was used to measure volume, was stored at -40°C until being assayed.

Assays Plasma and urine concentrations of VPA, 3-keto VPA, 3-OH VPA, and VPA-glucuronide were determined using the GC-MS method with selected-ion monitoring (QP-5000, Shimadzu, Japan). Extraction and trimethylsilyl derivatization procedures were similar to those published by Tatsuura *et al.*,¹⁸⁾ but ethyl acetate was used as the extraction solvent and undecylenic acid was used as the internal standard (I.S.). The following ions were selected: m/z 201 (VPA), m/z 242 (I.S.), m/z 275 (3-OH VPA), m/z 287 (3-keto VPA). For the assay of the total VPA (free plus conjugate), urine or plasma was subjected to base hydrolysis.¹⁸⁾ The VPA glucuronide concentration was obtained by subtracting the free VPA concentration from the total VPA concentration.

RESULTS

The plasma concentration of VPA was in the therapeutic range (50–100 $\mu\text{g/ml}$) (Fig. 2). The concentration of the metabolites was lower than those of VPA. Blood samples were not taken in patient Y.T. and F.T. during the experimental period.

Table 2. Urinary Excretion of Valproic Acid and Its Metabolites in the Epileptic Patients^{a)}

Patient	VPA (%)	3-OH (%)	3-keto (%)	Glu-VPA (%)	Recovery (%)
Y.T. (1280)	0.1	1.1	21.5	20.4	43.1
K.S. (1740)	0.2	0.9	13.1	20.5	34.7
F.T. (1920)	0.1	1.5	26.2	13.1	40.9
N.T. (1410)	0.2	1.0	18.7	30.8	50.7
I.T. (1540)	0.4	0.7	5.8	88.7	95.6
N.S. (650)	0.5	0.6	12.0	46.9	60.0

Each value represents percentage of dose. The values in the parentheses are urine volume (ml/24 h). a) 24-h-urinary excretion.

Table 2 lists the urinary excretion of VPA and its metabolites. The urinary recovery of VPA and its metabolites over 24 h was from 34.7% to 95.6% of the dose administered. The amount of VPA and 3-OH VPA excreted in the urine was low: 0.1–0.5% and 0.6–1.5% of the dose of VPA administered, respectively. The amount of 3-keto VPA and glucuronide (VPA-Glu) was marked: 5.8–26.2% and 13.1–88.7% of the dose of VPA, respectively. Patients I.T. and N.S. took a relatively large amount of VPA (2.8 g/d) and produced a high urinary excretion of VPA-Glu.

DISCUSSION

VPA is highly bound to plasma proteins (*ca.* 90%) in a therapeutic range and the hepatic extraction ratio is low. The liver eliminates VPA, considering that VPA is metabolized by β -oxidation, glucuronidation, *etc.* Its water soluble metabolites are subsequently excreted in the urine. The epileptic patients who participated in this study had normal hepatorenal function considering their laboratory values (data not shown).

The plasma concentration of VPA metabolites (3-keto VPA, 3-OH VPA and VPA-Glu) in epileptic patients at a steady state was lower than that of VPA (Fig. 2). These results were consistent with others.^{18,20,21)} The plasma concentration of VPA was in the therapeutic range. However, the concentration might exceed the upper limit of the therapeutic range (100 $\mu\text{g/ml}$) in patients N.T. and N.S. 1–2 h after administration. Patient N.T. (plasma half life of VPA; *ca.* 10 h) took a sustained-release preparation (Selenica R, t_{max} 6–12 h), so that the concentration did not exceed 100 $\mu\text{g/ml}$. On the other hand, patient N.S. took a general tablet (Depakene), and the half life of VPA in the patient was short (*ca.* 7 h). Al-

though the concentration in patient N.S. could exceed 100 $\mu\text{g/ml}$, the patient showed no side effects. Clinically, VPA can be administered over 100 $\mu\text{g/ml}$ to treat the seizures unless side effects appear. Bruni *et al.* reported that a relatively high concentration (140 $\mu\text{g/ml}$) of VPA was effective without symptoms of poisoning.²²⁾

Hyperammonemia is common in patients who take VPA, but does not necessarily cause hepatic damage. The ammonia concentration increase is probably due to the inhibitory effect of VPA on mitochondrial function, not to the VPA metabolites containing 4-en-VPA, a hepatotoxic metabolite.^{23,24)} Patients I.T. and N.S. took a relatively large dose, 2.8 g/d compared to 0.4–1.2 g/d, to maintain an effective VPA plasma concentration. Their plasma concentrations of ammonia (about 100 μM) were a little higher than the normal range (12–84 μM), but they showed no symptoms.

Tatsuhara *et al.* reported that the urinary recovery of VPA metabolites was about 60% (for 120 h) in healthy male volunteers after a single oral administration, and 3-keto VPA, VPA-Glu, and 3-OH VPA were excreted 37.7, 10.1, and 3.4%, respectively.¹⁵⁾ Anderson *et al.* reported that almost 80% of the VPA metabolites were recovered (3-keto VPA, 36%; VPA-Glu, 30%; 3-OH VPA, 1.5%) in the 12 h urine of the healthy male volunteers.¹⁷⁾ In both studies, a small percentage of VPA was excreted into the urine, probably due to reabsorption by the kidney.

There have been few controlled studies on the urinary excretion of VPA or its metabolites in epileptic patients. Schäfer *et al.* reported the urinary concentration of 4 metabolites (3-keto VPA, 4-OH VPA, 5-OH VPA, VPA-Glu) in 11 epileptic patients under various conditions (urine volume and collection time were inconsistent).²⁵⁾ They showed that the formation of 3-keto VPA and VPA-Glu was the normal route of VPA metabolism. Tatsuhara *et al.* measured the urinary excretion of 11 metabolites in 12 epileptic patients under various conditions.¹⁸⁾ They found that the oxidative products 3-keto, 2-propylglutaric acid (PGA), 3-OH VPA, and 4-OH VPA were major metabolites of VPA. They also showed that VPA and its unsaturated metabolites (Z)-2-en VPA and (E)-2-en VPA were mainly excreted as glucuronides in the patient's urine.

Levy *et al.* reported the mean urinary profiles of VPA and its 15 metabolites in epileptic patients under control conditions, but gave no description of the urine collection duration in the patients.²⁶⁾ They showed that the urinary metabolites accounted for a large majority of the dose: VPA monotherapy, 85.1 $\pm$ 19.5%; VPA and carbamazepine, 74.2 $\pm$ 20.9%; VPA and phenytoin, 71.4 $\pm$ 16.4%.

In this study, urine collection was controlled. The 24 h-urinary recovery of VPA and the 3 metabolites (calculated in the same manner as VPA) was quite different (34.7–95.6% of the dose administered). The patients predominantly excreted 3-keto VPA and VPA-Glu. Although 40 % of the dose administered may be excreted as glucuronide,⁶⁾ the urinary excretion of VPA-Glu in patients I.T. and N.S. was 88.7% and 46.9% of the dose administered, respectively. These values were marked compared to the values for healthy volunteers, especially in patient I.T. Although alkaline hydrolysis and β -glucuronidase hydrolysis could be used alternatively for the determination of VPA-Glu,¹⁵⁾ β -glucuronidase hydrolysis was additionally performed on the patient's urine to ver-

ify the results. The results obtained from the β -glucuronidase hydrolysis assay were similar to the alkaline hydrolysis assay.

Patients I.T. and N.S. excreted a high percentage of VPA-Glu, but the cause remains unknown. We hypothesize that co-administered drugs affect metabolism, but this possibility is low because the other patients also took other drugs. Although Levy *et al.* reported that the formation clearance of the VPA conjugation pathway was significantly greater in the VPA and phenytoin group than in the VPA monotherapy group, phenytoin was not believed to induce UDP-glucuronosyl transferase (UGT).²⁶⁾ Also, we hypothesize that the saturation of oxidative metabolism occurred due to the relatively large dose administered (2.8 g/d). The percentage of 3-keto VPA excreted in the urine was lower in patients I.T. and N.S. compared with the other patients, therefore, the β -oxidation might have been saturated. Patients I.T. and N.S. eliminated the normal dose of VPA rapidly, and it was difficult to explain this phenomenon by saturation of oxidative metabolism. Lastly, we hypothesize that there was an increase in glucuronidation. If the hepatic UGT activity was high, then VPA-Glu would be in the plasma at detectable concentrations. The plasma concentration of VPA-Glu was not detected in the patients as described above, but the blood sampling was not done in 2 patients.

In conclusion, the urinary excretion of VPA and its metabolites by patients who have taken a normal amount of a VPA preparation was similar to that of healthy volunteers. However, two epileptic patients who took a relatively large amount of the VPA preparation excreted VPA-Glu without an increase of VPA-Glu in the plasma. It might be clinically useful to determine the urinary VPA-Glu of the patients who need to take a relatively large dose of VPA, in order to understand why the plasma VPA concentration did not readily increase in these patients and to develop a better treatment for the patients.

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