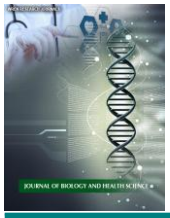

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Research Article

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Monitoring of Sodium Valproate and Its Metabolite 2-Propyl-2-Pentenoic Acid in Blood and Analysis of Factors Influencing Hepatotoxicity

Li Longkuan[1], Luo Huan[1], Zhang Wei[2], Jiang Jing[2] and Meng Fuxue[1*]

[1]Department of Pharmacy, Third Affiliated Hospital of Guizhou Medical University, Duyun, China
[2]Department of Psychiatry, Third Affiliated Hospital of Guizhou Medical University, Duyun, China

***Correspondence:** Meng Fuxue, Department of Psychiatry, Third Affiliated Hospital of Guizhou Medical University, Duyun, China, E-mail: mfx5675143@126.com; DOI: https://doi.org/10.56147/jbhs.2.6.94

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Abstract

Objective: To investigate factors affecting the attainment of VPA plasma concentration targets in pediatric epilepsy patients and to analyze the relationship between 2-ene-VPA plasma concentration and VPA plasma concentration, dosing regimen, as well as liver and kidney function parameters.

Methods: Based on inclusion and exclusion criteria, retrospective analysis was conducted on pediatric epilepsy patients treated at our hospital. VPA and its metabolite 2-ene-VPA plasma concentrations were measured, along with basic patient information and biochemical indicators such as liver and kidney function. Potential factors influencing VPA plasma concentration attainment were analyzed.

Results and conclusion: (1) Statistically significant differences in VPA plasma concentration were observed among the VPA group, VPA combined with LEV group, VPA combined with TPM group and VPA combined with LTG group ($F=17.27$, $P<0.05$). No statistically significant differences were found in 2-ene-VPA plasma concentration ($F=0.045$, $P>0.05$); There was no significant difference in VPA blood concentration between the two groups ($P>0.05$); (2) The dosage and formulation of VPA significantly influenced the achievement of target blood concentration ($P<0.05$). Logistic regression analysis revealed that the formulation, dosage and concomitant use of Topiramate (TMP) also had statistically significant effects on VPA blood concentration ($P<0.05$); (3) (1) In the VPA oral solution group, there was a correlation between VPA blood concentration and 2-ene-VPA blood concentration ($P=0.035$, $R=0.176$), while no correlation was observed in other groups; (2) The correlation between VPA dosage and blood concentration was low: VPA oral solution group ($P<0.001$, $R=0.483$), VPA sustained-release tablet group ($P<0.001$, $R=0.516$) and 15-30/(kg·d) dosage group ($P<0.001$, $R=0.305$); (3) No correlation was found between VPA dosage and 2-ene-VPA blood concentration; (4) VPA blood concentration showed correlations with Creatinine (CREA) ($P<0.001$, $R=0.269$), Total Protein (TP) ($P<0.001$, $R=-0.158$) and Globulin (GLB) ($P<0.001$, $R=-0.229$).

Conclusion: Sodium valproate exhibits significant individual variations in blood concentration. Dosage and formulation are critical factors affecting VPA blood concentration attainment. Clinical monitoring of blood concentration, Alanine Aminotransferase (ALT) and creatinine levels is essential.

Keywords: Sodium valproate; 2-propyl-2-pentenoic acid; Liver injury; Plasma concentration; Logistic regression

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Introduction

Valproic Acid (VPA), a first-line broad-spectrum antiepileptic drug, is primarily used in clinical practice to treat generalized and partial epilepsy, as well as manic episodes associated with bipolar disorder. Metabolized predominantly in the liver, VPA undergoes three main pathways: (1) Mitochondrial β-oxidation (40% of total metabolism), which primarily produces 2-Propyl-2-Pentenoic Acid (2-ene-VPA); (2) Glucuronidation and excretion (50%); and (3) Cytochrome P450-mediated metabolism (10%), generating 2-Propyl-4-pentenoic Acid [1]. VPA exhibits significant individual variations in dosage and blood concentration, with a nonlinear dose-response relationship. Blood levels are influenced by multiple factors and adverse reactions mainly include gastrointestinal effects such as nausea, vomiting, diarrhea and abdominal pain, while liver damage remains one of its most severe side effects [2].

This study retrospectively analyzed the treatment outcomes of pediatric epilepsy patients receiving VPA for over one month, measuring blood concentrations of VPA and its metabolite 2-ene-VPA. It explored the relationship between these drug levels and biochemical indicators such as liver and kidney function in the patients. The research also examined the effects of concomitant antiepileptic drugs (*e.g.,* levetiracetam, lamotrigine and topiramate) on VPA and 2-ene-VPA concentrations, identifying potential factors affecting VPA's therapeutic target levels. These findings provide a reference for the clinical safe and rational use of VPA.

Materials and Methods

Research objects

This study enrolled pediatric patients diagnosed with epilepsy at our hospital outpatient department between January 2022 and December 2023, who were receiving treatment with valproic acid sustained-release tablets or oral sodium valproate solution. The study also included patients concurrently treated with Levetiracetam (LEV), Lamotrigine (LTG) or Topiramate (TPM). The protocol was reviewed by the Ethics Committee of the Third Affiliated Hospital of Guizhou Medical University, with approval number: 2022H006. Inclusion criteria were: (1) Children diagnosed with epilepsy according to the clinical diagnosis and treatment guidelines-epilepsy volume; (2) Children undergoing routine blood drug concentration monitoring; (3) Age between 0-18 years; (4) Normal liver and kidney function; and (5) Children who could adhere to medical instructions for at least one month of VPA administration. Exclusion criteria included: (1) Children with other severe comorbidities and; (2) Children unable to follow medical medication regimens.

Blood sample collection and clinical data collection

According to the inclusion criteria, venous blood of 2 mL was obtained from the patient before morning medication, anticoagulated with EDTA and centrifuged at 3000 r/min for 5 minutes. Plasma was then collected for routine VPA drug concentration measurement and the plasma samples were frozen and stored at-80°C. The patient's name, gender, age, ethnicity, VPA monitoring results, concomitant medication and liver function indicators were recorded: Alanine Aminotransferase (ALT), Total Bilirubin (TBIL), Direct Bilirubin (DBIL), Indirect Bilirubin (IBIL), Total Protein (TP), Albumin (ALB), Globulin (GLB) and Albumin/Globulin ratio (A/G). Renal function indicators included Urea Nitrogen (BUN), Creatinine (CREA), urea/creatinine ratio and serum cystatin C (Cys C). Liver injury was assessed according to the China guidelines for the diagnosis and treatment of drug-induced liver injury (2023 edition), with the target concentration of sodium valproate being 40 μg/m-100 μg/m.

The blood concentration of 2-ene-VPA, a metabolite of VPA, was determined using the detection method established by the research team [3]. Chromatographic conditions: Agilent ZORBAX SB-C18 liquid chromatography column (4.6 mm × 150 mm, 5 μm), mobile phase: methanol-water (55:45), flow rate: 1 mL/min, column temperature: 35°C, injection temperature: 20°C, UV detection wavelength: 217 nm, injection volume: 20 μL.

Equipment and reagents

The analysis was performed using a Roche Cobas c311 fully automated biochemical analyzer, Agilent 1260 infinity High-Performance Liquid Chromatography (HPLC) system, ChemStation edition for LC & LC/MS systems C.01.04 data processing system and AL204 electronic analytical balance. Reagents included: 2,4'-dibromophenylacetone (Aladdin, batch no. 19703); propionic acid detection kit (homogeneous enzyme immunoassay); propionic acid sodium sustained-release tablets (Xindong Biotechnology Co., Ltd., batch no. 6DB1911; Sanofi Hangzhou Pharmaceutical Co., Ltd., DHG1367, EHG0059); propionic acid sodium oral solution (Sanofi Hangzhou Pharmaceutical Co., Ltd.; specifications: 300 ml, 12 g; batch no. EHG0356, DHG0633); 2-ene-VPA reference standard (Toronto Research Chemicals Inc., Toronto, Canada, batch no. 172108); chromatography pure methanol; other reagents were analytical grade; and water was double distilled.

Data processing and analysis

Statistical analysis was performed using SPSS 26.0 software. Quantitative data were presented as means, with one-way ANOVA used for inter-group comparisons. For statistically significant differences, pairwise comparisons

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were conducted using the Student-Newman-Keuls (SNK) method. Categorical data were expressed as proportions or rates and χ^2 tests were employed for inter-group comparisons. Bivariate correlation analysis was performed to examine the relationships between VPA dosage and VPA blood concentration, 2-ene-VPA blood concentration, as well as between VPA and 2-ene-VPA concentrations. If data followed a normal distribution, linear correlation analysis was performed; otherwise, rank correlation analysis was used.

A binary logistic regression model was constructed using the backward LR method to identify factors affecting VPA blood concentration attainment. A P value ≤ 0.05 was considered statistically significant, while a P value>0.05 indicated no significant difference.

Results

Basic information of the patients and monitoring results of VPA and 2-ene-VPA blood concentrations

This study enrolled 247 children with epilepsy, including 157 males and 90 females, aged 0-18 years (mean age: 7.28 ± 4.86 years). The mean VPA dosage was 19.02 ± 6.52 mg/kg, with a mean blood concentration of 61.89 ± 30.34 μg/mL. The treatment compliance rate was 63.97%. The mean 2-ene-VPA concentration was 3.84 ± 1.60 μg/mL. Statistical differences were observed in VPA blood concentrations between the VPA group, VPA+LEV group, VPA+TPM group and VPA+LTG group, while no significant difference was found in 2-ene-VPA concentrations **(Table 1)**.

Table 1: Basic information of pediatric patients and monitoring results of VPA and 2-ene-VPA blood concentrations *($\bar{x}$ ± s, n=247)*.

Group	Number of cases	Gender (male/female)	Age (years)	Ethnicity (Han/ minority)	VPA dosage/ (mg/kg)	VPA concentration (μg/mL)	2-ene-VPA concentration/ μg/mL
VPA	181	116/65	6.40 ± 4.51	121/60	17.94 ± 5.74	55.36 ± 26.35[b,c,d]	3.86 ± 1.56
VPA+LEV	32	20/12	10.31 ± 5.15	20/12	18.69 ± 5.84	68.44 ± 31.69[a,d]	3.80 ± 1.89
VPA+TPM	17	12/5	7.68 ± 4.79	14/3	23.00 ± 6.66	84.01 ± 31.23[a,d]	3.89 ± 1.79
VPA+LTG	17	9/8	10.62 ± 4.55	13/4	27.25 ± 8.34	99.79 ± 30.85[a,b,c]	3.73 ± 1.31
						F=17.27	F=0.045

Note: Compared with the VPA group (*vs.* VPA), [a]*P<0.05;* compared with the VPA+LEV group (*vs.* VPA+LEV), [b]*P<0.05;* compared with the VPA+TPM group (*vs.* VPA+TPM), [c]*P<0.05* and; compared with the VPA+LTG group (*vs.* VPA+LTG) and [d]*P<0.05*.

Distribution of abnormal liver and kidney function indicators in children with epilepsy

The patients were divided into four groups based on medication use. As shown in **Table 2**, the liver and kidney function indicators in each group exhibited abnormalities, with CREA showing the highest abnormality rate at 82.19%, followed by ALT at 13.77%.

Table 2 distribution of abnormal liver and kidney function indicators in children with epilepsy (n=247).

Table 2: The abnormal distribution of liver and renal index in 247 cases of epilepsy children.

Group	Renal function indicators			Liver function index							
	BUN	CREA	CysC	ALT	TBIL	DBIL	UBIL	TP	ALB	GLB	A/G
VPA	7	156	8	25	4	9	2	22	15	6	6
VPA+LEV	6	25	1	1	2	3	1	2	0	0	0
VPA+TPM	3	12	1	3	0	0	0	3	0	1	0
VPA+LTG	1	10	0	5	0	0	0	2	0	1	1
Amount to	17	203	10	34	6	12	3	29	15	8	7
Abnormal ratio	6.88%	82.19%	4.05%	13.77%	2.43%	4.86%	1.21%	11.74%	6.07%	3.24%	2.83%

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Correlation between VPA and 2-ene-VPA plasma concentrations and liver/renal function parameters

Pearson correlation analysis revealed significant relationships between VPA plasma Concentrations and Creatinine (CREA) (P<0.001, R=0.269), total protein (TP) (P<0.001, R=-0.158) and Globulin (GLB) (P<0.001, R=-0.229). 2-ene-VPA concentrations showed statistically significant linear correlations with TP and GLB, but no significant linear correlations with other parameters, as shown in **Table 3**.

Table 3: The correlation between VPA and 2-ene-VPA blood drug concentration and liver and kidney function indicators.

Biochemical parameters	Concentration	VPA/61.88 ± 30.34 (μg/mL)		2-ene-VPA/3.85 ± 1.60 (μg/mL)	
		P-value	Coefficient R	P-value	Correlation R
BUN	(4.40 ± 1.25) mmol/L	0.024	0.144	0.546	-0.039
CREA	(44.03 ± 16.46) μmol/L	<0.001	0.269	0.455	-0.048
BU/CREA	27.67 ± 12.26	0.034	−0.135	0.932	0.005
CysC	(0.87 ± 0.16) mg/L	0.419	−0.052	0.357	0.059
ALT	(14.20 ± 15.97) U/L	0.832	−0.014	0.336	0.061
TBIL	(9.86 ± 5.50) μmol/L	0.906	-0.008	0.985	-0.001
DBIL	(3.85 ± 2.02) μmol/L	0.068	0.29	0.514	-0.042
UBIL	(6.04 ± 4.09) μmol/L	0.644	−0.030	0.678	-0.232
TP	(69.82 ± 4.79) g/L	0.282	−0.069	<0.001	-0.158
ALB	(43.79 ± 3.16) g/L	0.104	−0.104	0.166	-0.088
GLB	(25.98 ± 3.78) g/L	0.918	0.007	<0.001	-0.229
A/G	1.72 ± 0.29	0.573	−0.036	0.004	-0.183

Results of the univariate analysis of VPA therapeutic drug monitoring target attainment single factor

Therapeutic drug monitoring target attainment Single factor analysis showed that the dosage and dosage form of VPA had a significant effect on the blood Concentration Achievement of VPA in children with epilepsy. The blood concentration of VPA was 40~100 μg/mL, as shown in **Table 4**.

Table 4: The univariate analysis of factors affecting the achievement of blood drug concentration standards in children with epilepsy.

Influencing factor	Divide into groups	Number of qualified individuals	Number of people below the required concentration level	Achievement rate/%	χ^2	*P*
Age	0~3 years	42	23	64.62	1.464	0.917
	4~6 years old	42	24	63.64		
	7~9 years old	30	18	62.5		
	10-12 years old	14	6	70		
	13~15	21	10	67.74		
	16 to 18 years old	9	8	52.94		
Sex	Man	95	62	60.51	2.235	0.135
	Woman	63	27	70		
Nation	The Han nationality	104	62	62.65	0.381	0.537
	Minority nationality	54	27	66.67		
VPA dosage	<15mg/(kg·d)	34	35	49.28	11.022	0.004
	15~30mg/(mg/kg)	111	52	68.1		
	>30 mg/(kg·d)	13	2	86.67		
The form of a drug	Oral solution	121	56	68.36	5.232	0.022
	Extended action tablet	37	33	52.86		
ALT	Normal	122	76	61.62	2.394	0.122
	Unusual	36	13	73.47		
CREA	Normal	26	18	59.09	0.552	0.457
	Unusual	132	71	65.02		

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	Share	21	11	65.63		
Share LEV	Incompatible	137	78	63.72	0.044	0.834
	Share	14	3	82.35		
Share TPM	Incompatible	144	86	62.61	2.677	0.102
	Share	11	6	64.71		
Share LTG	Incompatible	147	83	63.91	0.004	0.948

Logistic regression analysis

Taking the blood concentration of valproic acid as the dependent variable and taking age, sex, nationality, dosage of VPA, dosage form, ALT, CREA, combined with LEV, combined with TPM, combined with LTG as independent variables, the binary Logistic regression model was used to analyze the factors affecting the blood concentration of VPA **(Tables 5 and 6)**.

Table 5: Assignment of factors affecting the attainment of target blood drug concentration in children with epilepsy.

Influencing factor	Variable name	Quantized value
	y	Within the standard range = 0, outside the standard range =1
Age	X1	(0~3 years old)=0; (4~6 years old)=1; (7~9 years old)=2; (10~12 years old)=3; (13~15 years old)=4; (16~18 years old)=5;
Sex	X2	Male=0; Female=1
Nation	X3	Han = 0; Ethnic minority =1
VPA dosage	X4	0=<15mg/(kg·d); 1=15~30/(kg·d); 2 => 30/(kg·d)
The form of a drug	X5	Oral solution = 0; Sustained-release tablet =1
ALT	X6	Normal=0; Abnormal=1
CREA	X7	Normal=0; Abnormal=1
Share LEV	X8	Yes=0; No=1
Share TPM	X9	Yes=0; No=1
Share LTG	X10	Yes=0; No=1

Table 6: Results of binary Logistic regression analysis on the influence of sodium valproate on the standard blood concentration (G=25.897, P=0.00012).

Influencing factor	β	SE	Wald	P	OR	95% CI	
The form of a drug	0.792	0.308	6.625	0.01	2.208	1.208	4.035
<15mg/(kg·d)	-	-	13.772	0.001	-	-	-
15~30/(kg·d)	−1.038	0.315	10.89	0.001	0.354	0.191	0.656
>30/(kg·d)	−2.018	0.809	6.228	0.013	0.133	0.027	0.648
Merge TMP	1.605	0.701	5.247	0.022	4.977	1.261	19.644
Constant	−1.382	0.689	4.03	0.045	0.251	-	-

Correlation analysis of blood drug concentrations of VPA and 2-ene-VPA

Pearson's linear regression analysis showed no correlation between the blood drug concentrations of VPA and 2-ene-VPA, as shown in **Table 7**.

Table 7: Correlation analysis of blood concentration of VPA and 2-ene-VPA.

Group	Number of cases	VPA dosage (Mg/kg)	VPA concentration (μg/mL)	2-ene-VPA concentration (μg/mL)	P-value	Correlation R
VPA oral liquid	139	17.16 ± 5.54	50.76 ± 23.61	3.90 ± 1.62	0.035	0.176
VPA extended action tablet	42	20.38 ± 5.74	71.10 ± 29.81	3.65 ± 1.29	0.664	0.069
<15 mg/(kg·d)	69	12.56 ± 1.97	42.92 ± 23.87	3.59 ± 1.37	0.997	<0.001
15~30/(kg·d)	162	20.19 ± 4.07	65.97 ± 25.93	4.00 ± 1.69	0.051	0.154
>30/(kg·d)	16	35.03 ± 4.62	102.28 ± 41.47	3.42 ± 1.39	0.39	−2.31

Correlation between VPA dosage and VPA/2-ene-VPA concentrations

Pearson's linear regression analysis showed no correlation between VPA dosage and 2-ene-VPA blood

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concentration. However, a weak linear relationship was observed between dosage and VPA blood concentration in both oral solution and sustained-release tablet groups, as shown in **Table 8**.

Table 8: The correlation analysis of VPA dose with VPA and 2-ene-VPA concentration.

Group	Number of cases	VPA dosage (Mg/kg)	2-ene-VPA			VPA		
			2-ene-VPA concentration (μg/mL)	P-value	Correlation R	VPA potency (μg/mL)	P-value	Correlation R
VPA oral liquid	139	17.16 ± 5.54	3.90 ± 1.62	0.448	0.065	50.76 ± 23.61	<0.001	0.483
VPA extended action tablet	42	20.38 ± 5.74	3.65 ± 1.29	0.76	-0.49	71.10 ± 29.81	<0.001	0.516
<15 mg/(kg·d)	69	12.56 ± 1.97	3.59 ± 1.37	0.536	0.076	42.92 ± 23.87	0.059	0.229
15~30/(kg·d)	162	20.19 ± 4.07	4.00 ± 1.69	0.768	-0.23	65.97 ± 25.93	<0.001	0.305
>30/(kg·d)	16	35.03 ± 4.62	3.42 ± 1.39	0.893	0.036	102.28 ± 41.47	0.407	0.223

Discussion

Since its clinical introduction in the 1960s, Valproic Acid (VPA) has been recognized as the first-line treatment for generalized epilepsy and has become one of the most widely used antiepileptic drugs. Due to its histone deacetylase inhibitory effects, VPA has also been extended to treat certain types of cancer, Human Immunodeficiency Virus (HIV) infections and neurodegenerative diseases. However, its severe adverse effects, particularly hepatotoxicity, have raised significant concerns among clinicians.

This study collected blood drug concentration data of VPA and 2-ene-VPA, as well as liver and kidney function indicators from 247 children with epilepsy. Studies have shown that children with epilepsy under 2 years old are at relatively higher risk of acute liver injury when treated with VPA, with a reported incidence rate of 1/600 to 1/800, compared to approximately 1/20,000 in the general population [4]. In 2023, the China Guidelines for the Diagnosis and Treatment of Drug-Induced Liver Injury revised the biochemical criteria for acute DILI, requiring ALT ≥ 5 × ULN or ALT ≥ 3 × ULN, along with TBil ≥ 2 × ULN [5]. Failure to meet these threshold standards can be defined as drug-induced liver biochemical abnormalities. In this study, ALT abnormalities were observed in 13.77% of cases, including one case of a 1-year-old Han Chinese female child with epilepsy who showed ALT levels four times the ULN, which returned to normal after discontinuation of the drug. Xu Shansen et al. identified 4 cases of children with epilepsy having ALT levels more than 3 × ULN in an observational study of 1,507 cases [6]. Chen Xi et al. found in a VPA blood concentration monitoring study involving 119 cases that the incidence of liver injury caused by VPA was 42.6%, with the judgment standard being ALT ≥ 2 × ULN [7]. Linear regression analysis revealed significant correlations between VPA plasma concentrations and renal function indicators (creatinine, CREA, P<0.001, R=0.269), hepatic function indicators (total protein, TP, P<0.001, R=-0.158) and Globulin (GLB, P<0.001, R=-0.229). Dong Lulu et al. demonstrated that patients with low plasma albumin levels exhibited significantly higher VPA concentrations and abnormal liver function rates compared to those with normal albumin levels [8]. While this study conducted regression analysis between liver function indicators and VPA concentrations, the correlations were statistically insignificant. Previous research indicates that 2-ene-VPA, when induced by potent hepatic enzyme activators, increases the formation of 2,4-dien-VPA, thereby indirectly causing hepatotoxic effects. Our findings revealed statistically significant linear correlations between 2-ene-VPA concentrations and TP/GLB levels, while no significant linear relationships were observed with other indicators. In our group's previous studies, age (P=0.080) and concomitant Levocetirizine (LEV) use (P=0.940) showed no statistical significance in 2-ene-VPA concentration differences, whereas gender demonstrated statistical significance in variations of 2-ene-VPA concentrations among pediatric epilepsy patients (P=0.021). 2-ene-VPA is generated through mitochondrial β-oxidation. Mitochondrial dysfunction can impair the activity and function of β-oxidase enzymes, leading to impaired β-oxidation and subsequent hepatotoxicity. The expert consensus on psychiatric drug genomics testing (2025) recommends screening for specific gene (POLG, OTC, CSP1) variants in patients suspected of mitochondrial disorders when using sodium valproate to prevent severe adverse reactions [9]. However, in patients with mitochondrial disorders, the relationship between 2-ene-VPA (the primary mitochondrial β-oxidation metabolite of VPA) and VPA-induced adverse reactions still requires further basic and clinical research.

The sodium Valproate (VPA) package insert and existing literature indicate that therapeutic concentrations of VPA within the range of 40 μg/mL-100 μg/mL can still achieve therapeutic effects. Most current studies set the therapeutic concentration range for VPA at 50 μg/mL-100

μg/mL. In this study, clinicians observed that epilepsy patients who were taking medication normally but had VPA concentrations below the therapeutic range did not require dose adjustments. Through analysis of VPA's active metabolites, we found that 2-ene-VPA remained at elevated concentrations in patients with subtherapeutic VPA levels. This suggests that 2-ene-VPA may also exert antiepileptic effects, potentially explaining why seizures remained controlled despite subtherapeutic VPA levels. However, no clinical studies have reported the relationship between 2-ene-VPA blood concentrations and seizure control outcomes, either domestically or internationally. Based on practical considerations, this study established the therapeutic concentration range for VPA at 40 μg/mL-100 μg/mL for dose-response analysis. Univariate analysis of variance revealed that dosage form and administration route were significant factors affecting VPA blood concentration attainment (P<0.05), with the sustained-release tablet group showing higher compliance rates than the oral solution group (P=0.022). Logistic regression analysis demonstrated statistically significant differences in dose-response relationships between dosage form, administration route and concomitant topiramate use. Xu ZY found in a monitoring study of 1,142 VPA blood concentration cases that there was a statistically significant difference in average blood concentration between the oral solution group and sustained-release tablet group [10]. The daily dose per kilogram of body weight also showed statistically significant differences in its impact on VPA blood concentration. Sha Biying et al. conducted a retrospective cohort study on 194 children aged 1 to 14 years, discovering that the dosage form of VPA, daily medication dose and age were related factors affecting the achievement of VPA blood concentration targets, consistent with the results of this study [11]. Sodium valproate oral solution is rapidly absorbed in the body, while sodium valproate sustained-release tablets release the drug slowly in a non-exponential (first-rate, initially more than less) manner. The difference in pharmacokinetics between these two dosage forms leads to variations in blood concentration. This may explain why the oral solution group showed greater fluctuations in blood concentration compared to the sustained-release tablet group, resulting in different achievement rates. Pan Wen et al. found in a retrospective study on factors affecting sodium valproate blood concentration targets in hospitalized patients that the achievement rate gradually increased with higher VPA doses [12]. In this study, the achievement rates for low, medium and high dose groups were 49.28%, 68.10% and 86.67% respectively (P=0.004), showing statistically significant differences. Chen Y demonstrated that the combined use of lamotrigine had no effect on VPA blood concentration, consistent with this study [13].

In this study, Logistic regression analysis revealed that dosage was a significant factor affecting the attainment of target VPA blood concentration levels in pediatric patients. The dosage was divided into three groups, with two dummy variables included in the model. The results showed statistical significance (P=0.001), indicating that dosage influenced VPA concentration attainment. Specifically, the 15 mg/(kg·d)-30 mg/(kg·d) group showed statistically significant differences (P=0.001 or=0.354, 95% CI 0.027-0.648), while the>30 mg/(kg·d) group also demonstrated significant differences (P=0.001 or=0.133, 95% CI 0.191-0.656). These findings confirm that dosage is a critical factor in achieving optimal VPA blood concentration levels.

In this study, based on the results of one-way analysis and logistic regression analysis, there was a statistically significant difference in the effects of dosage form and dose on VPA concentration. Therefore, the study was divided into groups according to dosage form and dose to explore their relationship. In the VPA oral solution group, there was a correlation between VPA blood concentration and 2-ene-VPA blood concentration (P=0.035, R=0.176). However, in the VPA sustained-release tablet group, 15 (kg·d)-30 (kg·d) dose group and>30/(kg·d) dose group, Li Longkuan et al. showed that when patients took VPA alone, there was a correlation between VPA blood concentration and 2-ene-VPA blood concentration, but the correlation was not significant [14]. Wilimowska J, et al. found no correlation between VPA blood concentration and 2-ene-VPA blood concentration in 27 patients with epilepsy who overdosed on VPA (P=0.0539, R=0.1263) [15]. Amini-Shirazi et al. reported a correlation between VPA blood concentration and 2-ene-VPA blood concentration in 19 epileptic patients aged 17-30 (P<0.001, R=0.475) [16]. The inconsistent findings from different studies indicate that further in-depth investigation is needed to clarify this issue.

Chen H, et al. conducted a multiple linear regression analysis to investigate factors affecting VPA blood concentration in adults, identifying dosage and gender as significant predictors [17]. This study, focusing on pediatric epilepsy patients, further demonstrated that dosage significantly impacts VPA concentration attainment. To explore the relationship between dosage and concentration, we divided VPA dosages into three groups for analysis, revealing no correlation between dosage and 2-ene-VPA blood concentration. Our subsequent correlation analysis showed a weak relationship between VPA dosage and blood concentration. Amini-Shirazi et al. [16] reported no significant correlation (P>0.05, R=0.07) between VPA dosage and 2-ene-VPA concentration in 19 epilepsy patients, a finding consistent with our results.

Conclusion

This study initially investigated factors affecting the blood concentration of Valproic Acid (VPA) and its metabolite 2-ene-valproic acid (2-ene-VPA), analyzing the relationships between 2-ene-VPA levels and VPA

concentrations, dosing regimens, as well as liver and kidney function indicators, to provide further clinical guidance. 2-ene-VPA has been shown to exhibit stronger antiepileptic activity than VPA while also demonstrating sedative effects, though reports indicate it may possess hepatotoxicity and neurotoxicity [18-19]. Nagai et al. demonstrated that 2-ene-VPA has a half-life of (14.2 ± 5.4) hours [20]. Due to its prolonged cerebral retention and slower elimination compared to VPA, 2-ene-VPA may contribute to the development of Valproic acid Encephalopathy (VHE). Although multiple studies suggest that sodium valproate encephalopathy may relate to the long-term accumulation of 2-ene-VPA in the brain and its interaction with intracerebral receptors, this hypothesis remains unconfirmed and requires further investigation.

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