Original article

Association between serum carnitine, ammonia and valproic acid levels in bipolar disorders patients

Saaya Yokoyama,[1] Norio Yasui-Furukori,[1,2] Taku Nakagami,[3] Kensuke Miyazaki,[4] Masamichi Ishioka,[5] Natsumi Tarakita,[6] Kazutoshi Kubo,[7] Norio Sugawara,[1] Kazutaka Shimoda[1]

[1]Department of Psychiatry, Dokkyo Medical University School of Medicine, Mibu, Tochigi, Japan
[2]Department of Neuropsychiatry, Hirosaki University Graduate School of Medicine, Hirosaki, Aomori, Japan
[3]Department of Psychiatry, Nakagami Mental Clinic, Ohdate, Akita, Japan
[4]Department of Neuropsychiatry, Hirosaki-Aiseikai Hospital, Hirosaki, Aomori, Japan
[5]Department of Psychiatry, Minato Hospital, Hachinohe, Aomori, Japan
[6]Department of Mental Health, Mutsu General Hospital, Mutsu, Aomori, Japan
[7]Department of Neuropsychiatry, Ohdate General Hospital, Ohdate, Akita, Japan

Correspondence should be addressed to: Norio Yasui-Furukori, MD, PhD
Department of Psychiatry,
Dokkyo Medical University School of Medicine
Mibu, Tochigi 321-0293, Japan
Tel.: 81-282-86-1111
Fax: 81-282-86-5187
e-mail: furukori@dokkyomed.ac.jp

Abstract

Purpose Valproic acid (VPA) is regarded as not only an antiepileptic drug but also a mood stabilizer in bipolar disorder. Long-term VPA therapy can cause carnitine deficiency, which may result in an increase in the blood ammonia level in patients with epilepsy. However, there is little information about this effect in bipolar disorder patients. The aim of this study is to investigate the associations between serum VPA levels and carnitine and ammonia levels in psychiatric patients other than child patients with epilepsy.

Methods The subjects were 182 consecutive Japanese adult patients (mean age 54.3±19.5 years) diagnosed with bipolar disorder and treated with VPA. The serum VPA level, carnitine fraction, and plasma ammonia level were measured. The free carnitine and acylcarnitine fractions were measured using an enzyme cycling method.

Results Sixty-nine patients (38%) had low free carnitine levels. There were significant differences in sex, height, VPA dose, serum VPA levels, total carnitine, acyl carnitine, and the acyl/free carnitine ratio between patients with low free carnitine levels and those with values in the normal range. Simple and multiple regression analyses revealed that the VPA dose and serum VPA level were inversely and significantly correlated with free carnitine levels. Plasma ammonia levels were correlated with the VPA dose, serum VPA level and acyl carnitine level but not the free carnitine level.

Conclusion These findings suggest that carnitine deficiency is associated with VPA dose and serum VPA level in patients with bipolar disorder. However, it is unlikely that carnitine deficiency is associated with hyperammonemia in bipolar disorder patients.

Keywords

bipolar disorder, valproic acid, free carnitine, acyl carnitine, ammonia

Introduction

The World Federation of Societies of Biological Psychiatry (WFSBP) guidelines recommend valproic acid (VPA) as the only first-line mood-stabilizing medication, possibly reflecting concerns regarding the safety of lithium.[1] Overall, VPA is well tolerated with regard to weight gain, fatigue, cognitive function, gastrointestinal disturbances, tremors, hair loss, thrombocytopenia, and teratogenicity, although pancreatitis, hepatotoxicity, and hyperammonemia are infrequent adverse events.[2] VPA is, therefore, widely used in patients with not only epilepsy but also bipolar disorder.

VPA is extensively metabolized by the liver via glucuronic acid conjugation and mitochondrial beta- and cytosolic omega-oxidation, producing multiple metabolites, some of which may be toxic.[3,4] Metabolites of VPA, such as valproyl-CoA and 2-propyl-4-pentenoate and propionate, inhibit enzymes in the urea cycle, leading to elevation of the blood ammonia level.[5-8] Some studies have shown elevated ammonia levels in a large percentage of asymptomatic patients on VPA.[9-11] However, these findings mentioned above were based on data in epileptic patients, not bipolar patients.

In addition, carnitine is a carrier-type molecule required for the transport and oxidation of fatty acids in the mitochondria.[12] VPA inhibits the biosynthesis of carnitine by decreasing the concentration of alpha-ketoglutarate and may cause carnitine deficiency. The total and free plasma carnitine concentrations were significantly lower in patients taking VPA than in those not taking VPA.[13] Carnitine deficiency can cause several disorders by impairing fatty acid oxidation. In addition, carnitine deficiency may result in hyperammonaemia.[14-17] These findings were also based on data in epileptic patients, not bipolar patients.

However, there is little information on carnitine levels associated with VPA in patients

with psychiatric disorders, such as bipolar disorder. Generally, children have lower amounts of muscle than adults, indicating that they have a tendency for carnitine deficiency. Previous studies investigated an association between biochemical parameters and VPA in epilepsy patients, almost all of who were children.[9-17] The bipolar disorder patients evaluated in the present study were adults. A previous study reported that patients with various psychiatric conditions treated with polypharmacy including VPA had lower plasma carnitine levels than would be expected in their healthy counterparts,18 although that study did not examine the effect of free carnitine levels on hyperammonemia. In addition, the association between carnitine deficiency and hyperammonemia in patients with bipolar disorder remains unclear. The aim of this study is to investigate the associations between serum VPA levels and carnitine and ammonia levels in psychiatric patients other than child patients with epilepsy. Therefore, we examined the effects of serum VPA levels on carnitine and ammonia levels in a large sample of patients with bipolar disorder.

Methods

Subjects

This was a retrospective study conducted in 6 psychiatric hospitals and a psychiatric outpatient clinic from August 2018 to July 2019. The subjects were 211 consecutive bipolar disorder patients receiving sustained-release formulation of VPA monotherapy who all underwent examinations of VPA levels, ammonia levels and carnitine fractions. Twenty-nine patients diagnosed with conditions other than only bipolar disorder (comorbidity with epilepsy in 21 and moderate to severe intellectual disorders in 8) were excluded from the study, and 182 bipolar patients (24 inpatients and 158 outpatients) were

included in the study. They received benzodiazepine hypnotics in 125, benzodiazepine anxiolytics in 52, and laxatives in 36. Neither mood stabilizers nor antiepileptic drugs were administered. No patients suffered from poor nutritional condition. This observational study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the ethics committee of each hospital. The need for informed consent was waived via the opt-out method on each hospital website. In Japan, we were able to quantify not only VPA concentration and ammonia level but also serum carnitine fraction and total and free carnitine levels using the Japanese health insurance service.

Venous blood samples were obtained from the patients 2-6 h after a meal (9AM-1PM) before morning dosing of VPA. For the determination of the ammonia level, VPA level and carnitine fraction, a blood sample was withdrawn by arm venipuncture and collected into a tube containing EDTA and a plain tube. To ensure sample stability, the EDTA tubes were immediately taken by refrigerated transport to the laboratory for immediate determination of the ammonia level (within 30 minutes after the blood sample was taken). For the determination of VPA level and the carnitine fraction, blood samples were centrifuged, and the serum was stored at -20°C until analysis. The measurements of the plasma ammonia level, serum VPA level, and serum free and acyl carnitine levels were performed in the SRL Laboratory (Hachiohji city, Japan).

The serum VPA levels were quantified by enzyme immunoassay (EIA) (EMIT2000 Valproic acid assay, Siemens Healthcare Diagnostics Co. Japan). The detection limit for VPA levels was 1μg/ml. The inter- and intra-assay coefficient of variation (CV) values for VPA levels were less than 5.0%. Total and free carnitine levels were measured using the enzyme cycling method (KDK-1201, KAINOS Co, Tokyo). The detection limits of

total and free carnitine levels were 1μmol/ml. The inter- and intra-assay CV values for total and free carnitine were less than 5.0%. The acyl carnitine levels were calculated from the total minus the free carnitine levels. The ammonia levels were quantified by a modified Fiji-Okuda method (Ammonia Test Wako, Fujifilm Wako Chemical Co. Japan).[19] The inter- and intra-assay CV values for ammonia levels were 2.51 and 1.43%. At our hospitals, the normal ranges of plasma ammonia and total, free and acyl carnitine are 30-80 μg/dl, 45-91 μmol/L, 36-74 μmol/L, and 6-23 μmol/L, respectively. The characteristics of the subjects are shown in Table 1. The data are presented as the means ± standard deviations (Table 1).

Statistics

In the present study, comparisons of several factors between patients with and without low levels of free carnitine were performed using t-tests and chi-square tests. The data are presented as the means ± standard deviations. A p-value <0.05 indicated statistical significance. Univariate regression analyses were performed, and a single-tailed p-value <0.025 indicated statistical significance. The factors associated with serum levels of carnitine were examined using multiple regression analyses with forced entry; they included age, sex, body weight, serum VPA level and duration of VPA use. The factors associated with ammonia levels were examined using multiple regression analyses with forced entry; they included age, sex, body weight, serum VPA level, duration of VPA use, the free carnitine level, the acyl carnitine level and the acyl/free carnitine ratio. The dummy variables used were as follows: male=1, female=0. A p-value <0.05 indicated statistical significance. The SPSS Statistics software program for Windows, version 25.0 (IBM SPSS, Tokyo), was used for all analyses.

Results

The average (standard deviation) serum VPA level was 52.2±33.3 μg/ml. No adverse symptoms caused by VPA were observed in any patients. The clinical symptoms of bipolar disorder in all patients were stable.

The average (standard deviation) free carnitine level was 39.4±11.5 μmol/ml. Of the 182 patients, 69 (37.9%) had low levels of free carnitine: i.e. < 36 μmol/l. There were significant differences in sex, height, VPA dose, serum VPA levels, total carnitine levels, free carnitine levels, acyl carnitine levels, and the acyl/free carnitine ratio between patients with low levels of free carnitine and those with levels in the normal range (Table 1). Simple regression analyses showed that the free carnitine level was directly correlated with sex and weight and inversely correlated with serum VPA level, and multiple regression analysis found that the free carnitine level was directly correlated with sex and weight and inversely correlated with serum VPA level (Table 2).

The average (standard deviation) acyl carnitine level was 9.4±3.6 μmol/ml. Of the 182 patients, 26 (14.3%) had low levels of acyl carnitine: i.e. < 6 μmol/l. Simple regression analyses showed that the acyl carnitine level was directly correlated with age, weight and duration of VPA use (Table 2). Multiple regression analyses showed that the acyl carnitine level was directly correlated with age and weight (Table 2). Of the 182 patients, 16 (8.8%) had low acyl carnitine/free carnitine ratios. Simple and multiple regression analyses showed that the acyl carnitine/free carnitine ratio was directly correlated with age (beta=0.185, p=0.024) and inversely correlated with sex (beta=-0.183, p=0.015).

The average (standard deviation) ammonium level was 45.1±27.5 μmol/ml. Of the 182 patients, 14 (7.7%) had high ammonium levels, and 54 (30%) had low ammonium

levels. Simple regression analyses showed that the ammonium level was directly correlated with serum VPA and acyl carnitine levels, and multiple regression analyses revealed that the ammonium level was inversely correlated with the acyl carnitine level (Table 3).

Discussion

The results of this study showed that serum VPA levels were inversely correlated with free carnitine levels but not acyl carnitine levels. In addition, the serum VPA level was correlated with the ammonia level, although the significance disappeared with confounding factors. Therefore, VPA exposure led to carnitine deficiency and hyperammonemia in a serum level-dependent manner. When carnitine deficiency and hyperammonemia are present in patients treated with VPA, a dose reduction of VPA is needed, even in patients with bipolar disorders.

Although several studies with epilepsy patients indicated a significant decrease in free carnitine level and an inverse correlation between the VPA and free carnitine levels in patients taking VPA,[10,12-17] which is in line with our results, some reports failed to find an association between VPA therapy and the free carnitine level in patients with epilepsy.[11,20,21] Therefore, we conclude that VPA therapy may cause carnitine deficiency regardless of the underlying disease. No significant difference was found in the total or free carnitine levels between VPA-treated epileptic patients without neurological abnormalities or nutritional problems and age-matched controls.[12] It was suggested that VPA monotherapy did not result in decreases in free carnitine levels or in the accumulation of long-chain acylcarnitine.[9] Thus, the relationship between the use of VPA and serum free carnitine levels is complicated, and several factors influence the serum free carnitine levels in

patients treated with VPA, such as the use of other antiepileptic agents, nutritional state and age. Pediatric patients, patients with poor nutritional states or patients taking antiepileptic agents other than VPA were not included in our study. In addition, serum levels of VPA in this study in patients with bipolar disorders were at the lower limit of the therapeutic reference range for valproic acid, which is yet only established in epilepsy (50-100 μg/ml). Therefore, the discrepancy between previous studies with epilepsy and our study with bipolar disorders might be explained by this relatively lower than usual clinical setting in epilepsy or other studies using patients with epilepsy.

In the present study, VPA dose and serum VPA level were correlated with the ammonia level, although the significance disappeared in multiple regression analysis. Hyperammonemia is common, affecting 27.8% of epileptic patients taking VPA.[22] Although many patients are asymptomatic and the clinical significance of hyperammonemia associated with VPA therapy is still inconclusive, it may also rarely lead to hyperammonemia encephalopathy. The possible mechanisms might be related to an imbalance between ammoniagenesis and ammonia disposal in the urea cycle.[6] VPA inhibits the activity of carbamoyl phosphate synthetase I, the first enzymatic reaction in the urea cycle, thereby hindering the excretion of ammonia and increasing the plasma ammonia levels.[19]

Contrary to our expectation, the ammonia level was not correlated with the free carnitine level in this study. While some studies showed no association between free carnitine and ammonia levels in patients with epilepsy[23] and in patients with schizophnirea,[24] other studies found a negative correlation between serum ammonia and free carnitine levels in children with epilepsy.[11,13,16,20] In patients with bipolar disorders with and without hyperammonemia, the former patients had significantly lower free

carnitine and acyl carnitine levels.[17]

In addition, it is postulated that carnitine supplementation may increase the beta-oxidation of VPA, thereby limiting cytosolic omega-oxidation and the production of toxic metabolites that are involved in liver toxicity and ammonia accumulation. Not only carnitine deficiency but also hyperammonemia are improved by carnitine supplementation.[25] However, an observational study reported that the effect was observed in only half of the patients, and no change was found in the others.[26] Therefore, taking all evidence into consideration, definite conclusions regarding the association between the free carnitine and ammonia levels cannot be drawn. Disagreement regarding the relationship may be explained by the differences in age, disease and body status among sample populations in the existing studies.

This study has several notable limitations. First, it is limited by the determination of only free carnitine and acyl carnitine, although there are several other carnitine fractions.[11] The second limitation of this study was the lack of a clinical evaluation of patients who had hyperammonemia or carnitine deficiency in addition to their psychiatric symptoms. In addition, most data were trough VPA concentrations. However, although fluctuation of VPA concentration might be small due to sustained-release formulation,[27,28] the possibility that data of peak VPA concentrations were included in the study in some patients cannot be excluded because of real-world clinical setting. In addition, we have not known which concentration is responsible for its effect on carnitine concentration, yet. Finally, this study is limited by its cross-sectional design; as a result of this design, we could not determine causal relationships between VPA therapy and carnitine deficiency or hyperammonemia in our study population. A follow-up survey is needed to investigate these associations.

In conclusion, the results of this study suggest that carnitine deficiency is associated with serum VPA levels in patients with bipolar disorders. However, it is unlikely that carnitine deficiency is associated with hyperammonemia in bipolar disorder patients. Further studies are needed to confirm these associations in bipolar patients.

Acknowledgements

None

Authors' Individual Contributions

Saaya Yokoyama and Norio Yasui-Furukori designed the study and wrote the initial draft of the manuscript. Taku Nakagami, Kensuke Miyazaki, Masamichi Ishioka, Natsumi Tarakita, and Kazutoshi Kubo took samples and obtained patients' informed consents. Kazutaka Shimoda contributed to the analysis and interpretation of data, and Norio Sugawara assisted in the preparation of the manuscript. All other authors have contributed to data collection and interpretation and critically reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Role of funding source

This study was funded by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Research JSPS, 15H04754 (Principal Investigator Norio Yasui-Furukori). The funders had no role in the study design, data collection and

analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

Norio Yasui-Furukori has been a speaker for Otsuka Pharmaceutical Co., Ltd., Mochida Pharmaceutical Co., Ltd., Dainippon-Sumitomo Pharmaceutical Co., and MSD Co. Kazutaka Shimoda has received research support from Novartis Pharma K.K., Dainippon Sumitomo Pharma Co., Astellas Pharma Inc., Meiji Seika Pharma Co., Ltd., Eisai Co., Ltd., Pfizer Inc., Otsuka Pharmaceutical Co., Ltd., Daiichi Sankyo Co., and Takeda Pharmaceutical Co., Ltd., and honoraria from Eisai Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical Co., Ltd., Meiji Seika Pharma Co., Ltd., Janssen Pharmaceutical K.K., Shionogi & Co., Ltd., Dainippon Sumitomo Pharma Co., Daiichi Sankyo Co., and Pfizer Inc. The funders did not have any role in data collection or in the study design, analysis, decision to publish, or preparation of the manuscript. The remaining authors declare that they have no competing interests to report.

References

1. Yatham LN, Kennedy SH, Parikh SV, Schaffer A, Bond DJ, Frey BN, Sharma V, Goldstein BI, Rej S, Beaulieu S, Alda M, MacQueen G, Milev RV, Ravindran A, O'Donovan C, McIntosh D, Lam RW, Vazquez G, Kapczinski F, McIntyre RS, Kozicky J, Kanba S, Lafer B, Suppes T, Calabrese JR, Vieta E, Malhi G, Post RM, Berk M. Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) guidelines for the management of patients with bipolar disorder. Bipolar Disord 2018;20:97-170.
2. Nanau RM, Neuman MG. Adverse drug reactions induced by valproic acid Clin Biochem 2013;46:1323–38.
3. Ghodke-Puranik Y, Thorn CF, Lamba JK, Leeder JS, Song W, Birnbaum AK, Altman RB, Klein TE. Valproic acid pathway: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics. 2013;23:236-41.
4. Kondo T, Ishida M, Kaneko S, Hirano T, Otani K, Fukushima Y, Muranaka H, Koide N, Yokoyama M, Nakata S, et al. Is 2- propyl-4-pentenoic acid, a hepatotoxic metabolite of valproate, responsible for valproate-induced hyperammonemia? Epilepsia 1992;33: 550-554.
5. Vázquez M, Fagiolino P, Maldonado C, Olmos I, Ibarra M, Alvariza S, Guevara N, Magallanes L, Olano I. Hyperammonemia associated with valproic acid concentrations. Biomed Res Int. 2014:217269.
6. Aires CC, van Cruchten A, Ijlst L, de Almeida IT, Duran M, Wanders RJ, Silva MF. New insights on the mechanisms of valproate-induced hyperammonemia: inhibition of hepatic N-acetylglutamate synthase activity by valproyl-Coa. J Hepatol 2011;55:426-434.
7. Verbiest HB, Straver JS, Colombo JP, van der Vijver JC, van Woerkom TC. Carbamyl phosphate synthetase-1 deficiency discovered after valproic acid-induced coma. Acta Neurol Scand 1992;86:275-279.
8. Coulter DL. Carnitine, valproate, and toxicity. J Child Neurol 1991;6:7-14.
9. Adeva-Andany MM, Calvo-Castro I, Fernández-Fernández C, Donapetry-García C, Pedre-Piñeiro AM. Significance of l-carnitine for human health. IUBMB Life. 2017;69:578-594.
10. Verrotti A, Greco R, Morgese G, Chiarelli F. Carnitine deficiency and hyperammonemia in children receiving valproic acid with and without other anticonvulsant drugs. Int J Clin Lab Res 1999;29:36-40.
11. Nakajima Y, Ito T, Maeda Y, Ichiki S, Kobayashi S, Ando N, Hussein MH, Kurono Y, Sugiyama N, Togari H. Evaluation of valproate effects on acylcarnitine in epileptic

children by LC-MS/MS. Brain Dev 2011;33: 816-823.
12. Coppola G, Epifanio G, Auricchio G, Federico RR, Resicato G, Pascotto A. Plasma free carnitine in epilepsy children, adolescents and young adults treated with old and new antiepileptic drugs with or without ketogenic diet. Brain Dev 2006;28:358-65.
13. Hamed SA, Abdella MM. The risk of asymptomatic hyperammonemia in children with idiopathic epilepsy treated with valproate: relationship to blood carnitine status. Epilepsy Res 2009;86:32–41.
14. Thom H, Carter PE, Cole GF, Stevenson KL. Ammonia and carnitine concentrations in children treated with sodium valproate compared with other anticonvulsant drugs. Dev Med Child Neurol 1991;33:795–802.
15. Opala G, Winter S, Vance C, Vance H, Hutchison HT, Linn LS. The effect of valproic acid on plasma carnitine levels. Am J Dis Child 1991;145:999–1001.
16. Okumura A, Kurahashi H, Iwayama H, Numoto S. Serum carnitine levels of children with epilepsy: Related factors including valproate. Brain Dev 2019;41:516-521.
17. Maldonado C, Guevara N, Queijo C, González R, Fagiolino P, Vázquez M. Carnitine and/or acetylcarnitine deficiency as a cause of higher levels of ammonia. Biomed Res Int. 2016:2920108.
18. Moreno FA, Macey H, Schreiber B. Carnitine levels in valproic acid-treated psychiatric patients: a cross-sectional study. J Clin Psychiatry. 2005;66:555-558.
19. Okuda T, Fujii E. Colorimetric determination method of blood ammonia. Saishinigaku 1966;21:622-627.
20. Fung EL, Tang NL, Ho CS, Lam CW, Fok TF. Carnitine level in Chinese epileptic patients taking sodium valproate. Pediatr Neurol. 2003;28:24-27.
21. Fukuda M, Kawabe M, Takehara M, Iwano S, Kuwabara K, Kikuchi C, Wakamoto H, Morimoto T, Suzuki Y, Ishii E. Carnitine deficiency: Risk factors and incidence in children with epilepsy. Brain Dev. 2015;37:790-796.
22. Tseng YL, Huang CR, Lin CH, Lu YT, Lu CH, Chen NC, Chang CC, Chang WN, Chuang YC. Risk factors of hyperammonemia in patients with epilepsy under valproic acid therapy. Medicine (Baltimore). 2014;93:e66.
23. Hirose S, Hirata M, Azuma N, Shirai Z, Mitudome A, Oda T. Carnitine depletion during total parenteral nutrition despite oral L-carnitine supplementation. Acta Paediatr Jpn. 1997;39:194-200.
24. Ando M, Amayasu H, Itai T, Yoshida H. Association between the blood concentrations of ammonia and carnitine/amino acid of schizophrenic patients treated with valproic acid. Biopsychosoc Med 2017;11:19.
25. Maldonado C, Guevara N, Silveira A, Fagiolino P, Vázquez M. L-Carnitine

supplementation to reverse hyperammonemia in a patient undergoing chronic valproic acid treatment: A case report. J Int Med Res. 2017;45:1268-1272.

26. Nakamura M, Nagamine T. The Effect of Carnitine Supplementation on Hyperammonemia and Carnitine Deficiency Treated with Valproic Acid in a Psychiatric Setting. Innov Clin Neurosci. 2015;12:18-24.

27. Yasui-Furukori N, Saito M, Nakagami T, Niioka T, Sato Y, Fujii A, Kaneko S. Different serum concentrations of steady-state valproic acid in two sustained-release formulations. Psychiatry Clin Neurosci. 2007;61:308-312.

28. Fujii A, Yasui-Furukori N, Nakagami T, Niioka T, Saito M, Sato Y, Kaneko S. Comparative in vivo bioequivalence and in vitro dissolution of two valproic acid sustained-release formulations. Drug Des Devel Ther. 2009;2:139-144.

Table 1 Charateristics of subjects who had low and normal levels of free carnitine.

Factors	Low FC	Normal FC	p
Age (yo)	54.7 ± 20.5	53.3 ± 17.8	0.682
Sex (male, female)	27, 42	75, 38	>0.001
Height (cm)	159.3± 8.3	161.9 ± 8.5	0.045
Weight (kg)	60.6 ±14.0	63.7 ± 16.4	0.194
BMI (kg/m2)	23.9 ± 5.2	24.3 ± 6.1	0.626
VPA dose (mg/day)	673 ± 368	506 ± 251	>0.001
Duration of VPA dosing (M)	78 ± 54	82 ± 87	0.600
VPA levels (μg/ml)	60.1 ± 21.8	46.9 ± 37.6	0.009
Total carnitine levels (μmol/ml)	36.5 ± 8.1	56.5 ± 9.2	>0.001
Free carnitine levels (μmol/ml)	28.2 ± 6.4	46.4 ± 7.9	>0.001
Acyl carnitine levels (μmol/ml)	8.3 ± 3.7	10.1 ± 3.3	0.001
Acyl/free	0.31 ± 0.15	0.22 ± 0.08	>0.001
Ammonia levels	46.2 ± 35.0	43.7 ± 21.7	0.558

Data are means±standard deviations.

Abbreviations: FC, free carnitine; VPA, valproic acid; BMI, body mass index.

Table 2 Partial and multiple regression analyses of free carnitine and acyl carnitine levels in patients treated with VPA.

Factors	free carnitine		acyl carnitine	
	r	beta	r	beta
Age (yo)	-0.013	0.026	0.155*	0.243**
Sex (male 1, female 0)	0.312***	0.279***	0.072	0.023
Weight (kg)	0.189**	0.156*	0.161*	0.249**
VPA levels (μg/ml)	-0.194**	-0.170*	-0.091	-0.057
Duration of VPA dosing (M)	0.074	0.040	0.135*	0.103
R		0.390***		0.312**

*p<0.05, **p<0.01, ***p<0.001

Abbreviations: r, coefficient correlation; beta, partial correlation coefficient; VPA, valproic acid; R, multiple correlation coefficient

Table 3 Partial and multiple regression analyses of ammonia levels in patients treated with VPA.

Factors	r	beta
Age (yo)	-0.097	-0.033
Sex (male 1, female 0)	0.109	0.141
Weight (kg)	0.073	0.091
VPA levels (μg/ml)	0.149*	0.127
Free carnitine level (μmol/ml)	-0.097	-0.08
Acyl carnitine level (μmol/ml)	-0.183*	-0.168*
Duration of VPA dosing (M)	0.016	0.022
R		0.288*

*P<0.05

Abbreviations: r, coefficient correlation; beta, partial correlation coefficient; VPA, valproic acid; R, multiple correlation coefficient

Legend to Figure 1

Figure 1

Relationship between serum valproic acid levels, free carnitine, acyl carnitine, and ammonia levels. The thickness of the arrows is based on the effect size. Although previous studies suggest that hyperammonemia is caused by FC deficiency, no correlation between FC and ammonia levels was found in this study.

Abbreviations: VPA, valproic acid; FC, free carnitine; AC, acyl carnitine.