RESEARCH PAPER

Carnitine Deficiency in Chinese Children with Epilepsy on Valproate Monotherapy

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Objective: To explore the incidence and independent risk-factors of secondary carnitine deficiency in Chinese children with epilepsy on valproate monotherapy. **Methods:** The free carnitine and acylcarnitines levels in 299 children with epilepsy on valproate monotherapy between June 2014 and September 2015 were compared with age- and sex-matched 299 healthy controls. **Results:** Children with valproate monotherapy had lower free carnitine levels [23.86 (10.60) µmol/L] than the controls [36.37 (9.37) µmol/L] (P<0.01). Most acylcarnitines were significantly lower in children with valproate monotherapy than controls. 63 children (21.1%) with epilepsy had carnitine deficiency; 54 were asymptomatic. Female gender (OR 2.1), high alanine aminotransferase levels (OR 1.0) and long duration of VPA treatment (1-12 mo) (OR 1.9) were independent risk factors for secondary carnitine deficiency induced by VPA. **Conclusions:** Carnitine deficiency with valproate is more likely in females, those with transaminitis, and those receiving the drug for 1-12 months.

Keywords: *Acylcarnitine, Adverse-effects, Outcome, Toxicity*.

Valproate (VPA), the most widely prescribed antiepileptic drug (AED) worldwide [1] has a chemical structure similar to short chain fatty acids [2]. Carnitine is an amino acid derivative, which plays a very important role in the oxidation of fatty acids [3,4]. Carnitine deficiency is a metabolic state in which free carnitine in plasma is less than 20 µmol/L [5]. The effect of VPA on carnitine levels has been debated since long (6-11). In order to explore the incidence and independent risk factors of carnitine deficiency in children with VPA monotherapy, we measured the levels of free carnitine and acylcarnitines in Chinese children receiving VPA monotherapy and healthy controls.

Methods

Children with epilepsy (n=299) were enrolled at Beijing Children's hospital between June 2014 and September 2015. Inclusion criteria for patients were age 1 to 17 years; receiving valproate monotherapy; and treated with VPA for more than 1 month. Exclusion criteria for patients were: those on special dietary therapies such as ketogenic diet; with carnitine supplements; receiving steroid drugs; a diagnosis of progressive degenerative, musculoskeletal or metabolic diseases; and inhibition of intake of a varied diet. Age- and sex-matched 299 healthy subjects were recruited at the same hospital within the same period. Exclusion criteria for controls included those with carnitine-modifying healthcare products or only vegetarian diet.

The protocol was approved by the Ethical Committee of Beijing Children's hospital. Written informed consents were obtained from the participants and their guardians. Dry blood spots samples were collected. Free carnitine and acylcarnitines were analyzed by LC-MS/MS (AB SCIEX, United States).

Statistical analysis was carried out using SPSS 13.0 (Armonk, New York, USA). The data of free carnitine and acylcarnitines between patients and controls were analyzed using independent-samples t test. Logistic regression was used to evaluate the relevant factors to carnitine deficiency. The difference was considered statistically significant at P<0.05.

Results

The mean (SD) durations of VPA treatment in cases were 2.0 (1.1) years, the VPA dose was 410.7 (136.1) mg/day, and the VPA concentration was 62.5 (20.1) mg/L. The mean (SD) levels of ALT and AST in the children with epilepsy before valproate treatment were 15.93 (8.99) IU/L and 27.10 (6.58) IU/L, respectively. The mean (SD) levels of ALT and AST in controls were 16.10 (9.48) IU/L and 26.61 (7.48) IU/L, respectively. The mean (SD)

WHAT THIS STUDY ADDS?

- Children with epilepsy receiving VPA had low carnitine levels, especially females, those with transaminitis and with the duration of VPA treatment being 1month-12months.

levels were ALT [15.72 (10.63) IU/L], AST [27.15 (14.36) IU/L], Urea [4.5 (1.6) mmol/L], and Creatinine [36.8 (16.5) µmol/L] of the children with epilepsy after VPA monotherapy.

The cases presented significantly lower free carnitine levels [23.86 (10.60) µmol/L] than controls [36.37 (9.37) µmol/L] (P<0.01). Most acylcarnitines were changed in patients with VPA treatment (***Web Table* I**). The short chain acylcarnitines (except for C5DC) were significantly lower in the patients than in the control group. The ratio of short-chain acylcarnitines to free carnitine were significantly higher (P<0.01) in the patients. The ratio of medium chain acylcarnitine to free carnitine was not significant in the patients with VPA treatment and controls (P>0.05). The ratio of long-chain acylcarnitines to free carnitine was significantly higher (P<0.01) in the patients.

63 (21.1%) children with epilepsy were found to have carnitine deficiency; 54 of these were asymptomatic. Manifestations of carnitine deficiency observed in the remaining nine (8 females) included reversible weakness, hypotonia, and mental retardation, which were possibly related to lack of energy supply induced by carnitine deficiency.

The alternative risk factors in the multivariate logistic regression analysis included age, gender, durations of VPA treatment, urea concentration, creatinine concentration, ALT concentration, AST concentration and VPA trough concentration. Female gender, higher level of ALT serum concentration and the duration of VPA treatment (>1month, <12months) were the independent risk factors to secondary carnitine deficiency induced by VPA (***Table* I**).

DISCUSSION

In this study, we found low free carnitine concentration in patients with VPA monotherapy compared with controls; 63 children with epilepsy (21.1%) were found to suffer carnitine deficiency induced by VPA. In addition, we found the most acylcarnitines were significantly lower in the children with valproate monotherapy.

Our study was consistent with some studies which reported that VPA could induce the lower levels of free carnitines and acylcarnitines [9-11]. However, the study was limited by small sample size and the absence of baseline carnitine levels in epileptic children before VPA treatment, so we compared the levels of free carnitine and acylcarnitines between patients after VPA treatment and controls.

The possible reasons for differences could be as follow: (*i*) female patients prefer vegetarian diet not animal product in which L-carnitine is abundant; (*ii*) VPA was catalyzed to 4-pentenoic acid Valproate (4-ene VPA) which could reduce hepatotoxicity and reduced of synthesis of carnitines [12,13]; and (*iii*) the period of VPA treatment between 1 month and 12 months was key period that the important organs such as kidney and liver adapted to VPA treatment. The stimulation of VPA on kidney and liver possibly effected carnitine reabsorption and liver synthesis.

Although some patients suffering from carnitine deficiency were asymptomatic or had mild symptoms, some studies showed that long term carnitine deficient status could cause some serious consequences, even life-threatening complications such as encephalopathy. Therefore, diagnosis and treatment of carnitine deficiency without delay might be useful for patients [5,14-15]. We suggest that free carnitine and acylcarnitines of high-risk patients should be screened. In addition, we speculate that patients with carnitine deficiency associated with

TABLE I INDEPENDENT RISK-FACTORS OF CARNITINE DEFICIENCY ASSOCIATED WITH VALPROATE USE

Variable	*Odds Ratio (95%)*
Age ≤3y	1.9 (0.87-4.35)
[#]Female gender	2.1 (1.17-3.84)
[$#]Duration of treatment	1.9 (1.02-3.47)
*High VPA levels	1.0 (0.99-1.02)
[#]High ALT	1.0 (1.00-1.06)
High AST	1.0 (0.98-1.02)
High Urea	1.1 (0.95-1.38)
Higher Creatinine	1.0 (0.99-1.03)

*AST: aspartate aminotransferase; ALT: alanine aminotransferase; [#]P<0.05; *Valproate serum trough concentrations; [$]Duration of VPA treatment (>1 mo and <12 mo).*

valproate might be benefit from L-carnitine supplementation. However, further longitudinal studies are needed to confirm these findings.

Contributors: LQL: initiated the project and wrote the paper; SWQ: is the guarantor and carried out the study; JH: collected the clinical data. All authors contributed to the design and interpretation of the study and to further drafts.

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WEB TABLE 1 LEVELS (µMOL/L) OF ACYLCARNITINES IN PATIENTS RECEIVING VALPROATE AND CONTROLS

	VPA treatment, n=299	*Controls, n=299*	*P value*
C0	23.86 (10.60)	36.37(9.37)	<0.001
C2	15.59 (5.78)	16.85(5.26)	0.01
C3	1.55 (0.70)	1.79 (0.64)	<0.01
C4	0.25 (0.14)	0.27 (0.10)	0.01
C5	0.10 (0.04)	0.17 (0.06)	<0.01
C5DC	0.05 (0.02)	0.04 (0.02)	>0.05
C6	0.09 (0.04)	0.09 (0.03)	>0.05
C8	0.10 (0.06)	0.12 (0.07)	<0.001
C10	0.13 (0.07)	0.17 (0.10)	<0.001
C14	0.12 (0.20)	0.11 (0.04)	>0.05
C14:1	0.21 (0.21)	0.19 (0.14)	>0.05
C16	0.72 (0.32)	1.08 (0.33)	<0.001
C16OH	0.09 (0.12)	0.08 (0.10)	>0.05
C18	0.31 (0.17)	0.62 (0.18)	<0.001
C18OH	0.25 (0.12)	0.15 (0.07)	<0.001
Sum of C2-C5DC	17.49(6.22)	19.12(5.56)	<0.001
Short chain acyl-car/ free carnitine	0.79 (0.23)	0.54 (0.13)	<0.001
Sum of C6-C10	0.32 (0.15)	0.39 (0.19)	<0.001
Medium chain acyl-car/free carnitine	0.01 (0.01)	0.01 (0.01)	>0.05
Sum of C14-C18 OH	1.69 (0.56)	2.23 (0.59)	<0.001
Long chain acyl-car/ free carnitine	0.08 (0.03)	0.06 (0.02)	<0.001

C0: free carnitine; C2: acetylcarnitine; C3: propionylcarnitine; C4: butyryl- and isobutyrylcarnitine; C5:isovaleryl- and 2-methylbutyryl-carnitine; C5:1: tiglylcarnitine; C5DC: glutarylcarnitine; C6: hexanoylcarnitine; C8: octanoylcarnitine; C10: decanoylcarnitine; C14: myristoylcarnitine; C14:1: tetradecenoylcarnitine; C16: palmitoylcarnitine; C16OH: 3-hydroxypalmitoylcarnitine, C18: stearylcarnitine; C18OH: hydroxystearylcarnitine.