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The risk of asymptomatic hyperammonemia in children with idiopathic epilepsy treated with valproate: Relationship to blood carnitine status

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Summary
Purpose: Valproate (VPA) administration may be associated with adverse metabolic effects, among is hyperammonemia, which could suggest metabolic abnormalities as carnitine deficiency. This study aimed to evaluate the risk frequency of hyperammonemia and abnormal carnitine levels in children receiving VPA who were otherwise free of neurological or obvious nutritional problems.
Methods: Ammonia levels were prospectively evaluated in 60 epileptic children with primary epilepsy free of neurological or nutritional problems and were treated with VPA for at least 1 year. Forty healthy children were included as controls. Ammonia levels were correlated with total carnitine (TC), free carnitine (FC), acylcarnitine (AC) and AC/FC ratio. The abnormal ammonia and carnitine levels were also re-checked after 3 months treatment with L-carnitine.
Results: Compared to pre-treatment and control levels, the mean TC and FC were lower ($p < 0.001$) while ammonia ($p < 0.01$), AC ($p < 0.05$) and AC/FC ratio ($p < 0.01$) were higher. In the treated group of epileptics, TC and FC were negatively associated with ammonia ($r = -0.896$, $p < 0.0001$; $r = -0.935$, $p < 0.0001$). Significant associations were found between FC and AC/FC levels and patient's age (FC; $r = 0.457$, $p < 0.05$, AC/FC; $r = -0.435$, $p < 0.05$) and dose of VPA (FC; $r = -0.753$, $p < 0.001$, AC/FC; $r = 0.591$, $p < 0.01$). Ammonia was correlated with patients' age ($r = -0.532$, $p < 0.01$) and dose of VPA ($r = 0.673$, $p < 0.01$). The abnormal ammonia and carnitine levels were returned to normal after L-carnitine supplementation.

Abbreviations: AEDs, antiepileptic drugs; VPA, valproate; TC, total carnitine; FC, free carnitine; AC, acylcarnitine; AC/FC, acylcarnitine/free carnitine ratio; BMI, body mass index; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase.

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Conclusions: Epileptic children treated with VPA and free of neurological disabilities are at risk for hyperammonemia that may be associated with hypocarnitinemia. Patients will benefit from early recognition and preventive measures as carnitine supplementation.

Introduction

Valproate (VPA) is a commonly used broad-spectrum anticonvulsant to treat generalized and focal epilepsies in children. Long-term VPA administration is associated with adverse metabolic effects, among is hyperammonemia. Asymptomatic hyperammonemia (without encephalopathy or manifestations of hepatic failure) was frequently detected in epileptic patients on VPA (Altunbasak et al., 1997; Gidal et al., 1997). However, VPA-induced hyperammonemic encephalopathy is an unusual or infrequently reported complication characterized by dizziness, general malaise, drowsiness, lethargy, decreasing level of consciousness, vomiting, focal neurological deficits, cognitive slowing, uncontrolled seizures and generalized EEG slowing (Kifune et al., 2000; Verrotti et al., 2002; Panda and Radhakrishnan, 2004; Gerstner et al., 2006; Dealberto, 2007; Wadzinski et al., 2007; Chou et al., 2008).

Ammonia, the product of degradation of amino acids and other nitrogenated compounds, is a normal constituent of all body fluids. Its elimination is promoted by the urea cycle, which consists of a series of metabolic reactions in which the liver plays a fundamental role. Not, surprisingly, the development of hyperammonemia with VPA often raises concern among clinicians for the presence of any disturbance in urea cycle that results in an increase in intracellular and plasmatic ammonia. Some hypotheses have implicated direct hepatic damage by VPA metabolites (Kesterson et al., 1984; Sugimoto et al., 1987; König et al., 1994; Lheureux and Hantson, 2009), impaired hepatic metabolism of ammonia (Beghi et al., 1990; Panda and Radhakrishnan, 2004) and increased ammonia production by the kidneys (Warter et al., 1983) as causes of hyperammonemia induced by VPA.

As a branched chain carboxylic acid, VPA is extensively metabolized by the liver via glucuronic acid conjugation, mitochondrial β- and cytosolic omega-oxidation to produce multiple metabolites (4-ene-VPA and 2,4-diene-VPA), some of which may be involved in its hepatotoxicity (Lheureux and Hantson, 2009). Hyperammonemia has also been suggested to be a marker for other metabolic abnormalities as free fatty acids. It is considered as an indication for carnitine deficiency encountered in the same group of patients (Opala et al., 1991). In addition, carnitine deficiency has been linked to hepatic complications associated with VPA use (Murakami et al., 1990).

Carnitine is an amino acid derivative present in most human tissues particularly liver and muscles. It is mainly supplied from dietary amino acids, particularly meat and dairy products (Blass, 1989). However, it can be produced by endogenous synthesis in the liver and kidney from dietary methionine and lysine and by renal reabsorption (Vaz and Wanders, 2002). Carnitine is an essential cofactor required for the transport long-chain fatty acids across the mitochondrial membrane for β-oxidation, which results in energy production needed for metabolism. Carnitine is also involved in oxidation of ketone bodies, glucose and various amino acids (Borum and Bennett, 1986). It mops up certain acyl coenzyme A (acyl-CoA) groups and toxic intermediates that impair the citric acid cycle, urea cycle, pathways for gluconeogenesis and fatty acid oxidation from the mitochondria and peroxisomes during acute clinical crisis (Vaz and Wanders, 2002).

Carnitine deficiency occurs in primary inborn errors of metabolism as mitochondrial disorders, fatty acid oxidation disorders and glutaric aciduria (Angelini et al., 1992), nutritional problems (Borum and Bennett, 1986), defective renal reabsorption (De Vivo and Tein, 1990) and by intake of some medications as VPA (Opala et al., 1991; De Vivo et al., 1998; Verrotti et al., 1999; Bohan et al., 2001). The most important consequence of carnitine deficiency is impaired energy metabolism (Stumpf et al., 1985). Manifestations for carnitine deficiency include muscle weakness, hypotonia, nausea and vomiting, fatigue, recurrent infection, failure to thrive, poor appetite poor concentration, apathy and headache (Beghi et al., 1990; Shapira and Gutman, 1991). Data regarding acquired carnitine deficiency induced by antiepileptic medications are contradictory. It has been reported that carnitine concentrations were within normal range in patients treated with VPA but free of neurologic or nutritional problems (Hirose et al., 1998). Other studies reported that carnitine deficiency is not uncommon in epileptic patients in presence of severe neurologic disability and presence of other risk factors as polytherapy or nutritional deficiencies (Verrotti et al., 1999; Fung et al., 2003).

Objective: Data regarding the occurrence of hyperammonemia in children with epilepsy free of neurologic or nutritional problems and treated with VPA are controversial. This study aimed to evaluate the risk frequency of asymptomatic hyperammonemia (in presence or absence of abnormal liver enzymes) and abnormal carnitine levels in children receiving VPA who were otherwise free of neurologic complications or obvious nutritional problems. In practice, it is not routine to monitor ammonia levels in patients taking VPA. The correlations between ammonia levels and carnitine states were determined.

Study design

This prospective study included 60 children (mean age: 13.1 ± 4.2 years; male = 43, female = 17) with primary epilepsy recruited from the out-patient pediatric epilepsy clinic of the Neurology department of Assiut University Hospital, Assiut, Egypt. The protocol of the study was approved by the local ethical committee and all participants or their parents gave written informed consent before

participation. Epilepsy type was classified according to the recommendations of the International League Against Epilepsy (Commission on Classification and Terminology, 1989). The included patients were chosen based on the following criteria: (1) ethnic Egyptians, (2) normal growth, development, medical and neurological examination, (3) VPA was chosen as the treatment of choice for their epilepsy (mono- or polytherapy), (4) regular intake and compliance to VPA treatment for at least 1 year before inclusion in the study, and (5) normal liver function tests and ammonia level before participation. VPA was prescribed in recommended doses and according to the well-known guidelines. All treated patients gave history of compliance to AEDs and some of patients' compliance with medication was evaluated by measurement of blood VPA concentration. Clinical and laboratory evaluation of the patients were done, at diagnosis, after 1 year from starting VPA and after 3 months of L-carnitine therapy in the group of patients with abnormal ammonia or carnitine levels. Regular clinical follow up (monthly) was done for most of the patients as they were receiving their treatment through our clinic regularly supported by insurance resources. Patients who did not come at follow up were excluded ($n = 15$). The final analysis included a total of 60 patients. Patients were divided into 3 groups: group 1: before start of treatment with VPA ($n = 60$), group 2: after treatment with VPA by 1 year ($n = 60$) and group 3: after treatment with VPA and L-carnitine ($n = 38$). For comparison, 40 healthy children matched for age (12.5 ± 2.5 years), sex (male = 20, female = 20) and socioeconomic status were chosen as controls. Control subjects were recruited from the out-patient clinic of the pediatric department for treatment of minor non-specific condition (e.g. fever and sore throat). Neither any of the epileptic children nor control subjects had: (1) known organic acidemia or other metabolic disorder, liver disorder or pancreatitis, (2) malnutrition or history of poor dietary intake of food rich in carnitine as red meat and dairy products, and (3) history of recent treatment with antibiotics containing pivalic acid, which is known to reduce blood carnitine level (Diep et al., 1993).

Data collected per patient were the following:

(1) Biological variables including: age, gender, weight, height and body mass index (BMI) calculated as follows: BMI = weight (kg)/height (m^2).
(2) All participants were subjected to full medical and neurological history and examination to exclude developmental, medical, neurological or nutritional disorders.
(3) Seizures were analyzed determining seizure type, duration of illness, age at onset, type, duration and dose of AEDs and response to medications. According to the degree of control with AEDs, seizures were considered controlled if seizures were occasional or rare, but uncontrolled if the frequency of seizures was very frequent or frequent (Hamed et al., 2005). The frequency of seizures were divided as follows: (a) very frequent: seizures occurring several times a day or at intervals shorter than 7 days, (b) frequent: seizures at intervals longer than 7 days but shorter than 30 days, (c) occasional: seizures at intervals longer than 30 days but shorter than 1 year, and (d) rare: seizures at intervals longer than 1 year.

Specimen collection and analysis: For patients, blood samples were obtained two times (at diagnosis and after 1 year of treatment with VPA). A third sample was taken from patients with abnormal ammonia or carnitine levels ($n = 38$) at the end of 3 months of treatment with L-carnitine (100 mg/kg/day) to check for normalization of ammonia and carnitine levels. For control subjects, blood was withdrawn after regular breakfast by 2 h. For the treated group of patients (after 1 year of VPA treatment), blood was obtained by venipuncture without a tourniquet after regular breakfast was eaten by 2 h and before the administration of the morning dose of VPA therapy (Eyer et al., 2005). Each sample was divided into two tubes, one plain tube in which blood was allowed to clot at room temperature and centrifuged at 3000 rpm for 10 min and the serum was collected for the assay of AEDs levels, random blood glucose, kidney and liver function tests including serum total bilirubin, glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT). The second part of the blood was collected in a tube containing EDTA, placed on ice immediately and not allowed to stand for more than 15 min then centrifuged as soon as possible for separation of plasma to avoid false elevation of ammonia levels if the sample is left too long. If not analyzed immediately, aliquots were kept frozen at $-70\,^{\circ}C$. The plasma ammonia levels were determined using a Hitachi automatic analyzer by an enzyme assay method using glutamate dehydrogenase. Total carnitine (TC) and free carnitine (FC) plasma levels were determined by spectrometry according to the method described by Wan and Hubbard (1998). Acylcarnitine (AC) was calculated as the difference between TC and FC. The serum levels of AEDs were determined in the Therapeutic Drug Monitoring (TDM) lab, Assiut University Hospital, Assiut, Egypt, using fluorescence polarization immunoassay system of Abbott (EPIA), TDxFLX apparatus (Abbott Lab, Wiesbaden, Germany), as described before (Goma et al., 2004). The mean levels of lab biomarkers for the control group were as follows: ammonia: 36.43 ± 10.81 μg/dL, TC: 47.93 ± 3.71 μmol/L, FC: 40.87 ± 4.76 μmol/L, AC: 7.07 ± 2.34 μmol/L and FC/AC: 0.18 ± 0.07 μmol/L.

Statistical analysis: Calculations were done with the statistical package SPSS for windows, version 10 (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed using student's ''*t*'' or one way ANOVA tests as appropriate. The mean values of different variable were compared between patients and control groups using student's *t*-test for independent samples. Paired *t*-test was used to compare the mean values of biochemical variables before and after treatment. The associations between blood carnitine levels and different variables and risk factors were analyzed using simple linear regression.

Results

A total of 60 epileptic children (mean age: 13.1 ± 4.2) were included in this study. All participants had normal growth and development. All had regular diet with no obvious feeding problems. The demographic characteristics of the studied group are presented in Table 1. All patients had normal complete blood count, random blood glucose level and kidney function test.

Table 1 Demographic characteristics of the studied group.

Demographic data	Patients ($n=60$)	Controls ($n=40$)
Patients' age; years	6–18 (13.1 ± 4.2)	6–18 (12.5 ± 2.5)
Male/female	43/17	20/20
BMI	10.5–18.6 (15.8 ± 2.2)	12.0–22.0 (17.5 ± 3.5)
Age at onset; years	1–12 (4.3 ± 1.9)	–
Duration of illness; months	0.5–6.0 (4.7 ± 1.7)	–
Type of epilepsy:		
Generalized tonic–clonic	37 (61.7%)	–
Absence	7 (11.7%)	–
Myoclonic	4 (6.7%)	–
Focal	12 (20%)	–
AEDs utilized; # (%)		
VPA	43 (71.7%)	–
VPA + CBZ	17 (28.3%)	–
Drug dose, mg/day		
VPA	400–800 (605.6 ± 160.7)	–
SerumVPA level; μg/mL	50–100 (75.6 ± 15.5)	–
Degree of control on AEDs:		
Controlled	39 (65%)	–
Uncontrolled	21 (35%)	–

BMI: body mass index, AEDs: antiepileptic drugs, VPA (valproate), CBZ (carbamazepine).

Before treatment with VPA, patients did not show difference in biochemical variables compared to control group (Table 2). At the end of 1-year treatment with VPA, patients developed significantly abnormal levels of liver enzymes ($n=22$), ammonia ($n=12$) and carnitines ($n=38$). Hyperammonemia was reported in 20% ($n=12$). Plasma ammonia concentration was significantly elevated with VPA monotherapy or polytherapy ($p<0.05$), but higher in patients on VPA monotherapy ($p<0.05$) (Tables 2 and 3).

None of the patients demonstrated clinical manifestations of liver disturbance (i.e. asymptomatic hyperammonemia). A significant number of patients on VPA demonstrated abnormal carnitine states (levels and/or ratios) (63.4%, $n=38$) (monotherapy: 60.5%, $n=26$; polytherapy: 70.6%, $n=12$). None of the epileptic children before treatment demonstrated abnormal carnitine results (Tables 2 and 3). All patients with hyperammonemia had abnormal carnitine states ($n=12$) and elevated liver enzymes.

In the treated group of epileptics, TC was positively associated with FC ($r=0.884$, $p<0.0001$) and AC ($r=0.761$, $p<0.0001$) and negatively associated with ammonia ($r=-0.896$, $p<0.0001$). FC was positively associated with AC ($r=0.536$, $p<0.01$) but negatively associated with ammonia ($r=-0.935$, $p<0.0001$). Significant association was found between FC and AC/FC levels and patient's age (FC; $r=0.457$, $p<0.05$, AC/FC; $r=-0.435$, $p<0.05$), and dose of VPA (FC; $r=-0.753$, $p<0.001$, AC/FC; $r=0.591$, $p<0.01$). Ammonia was correlated with the patients' age ($r=-0.532$, $p<0.01$) and dose of VPA ($r=0.673$, $p<0.01$). Significant association was identified between ammonia levels and of SGPT ($r=0.530$, $p<0.01$) and SGOT ($r=0.435$, $p<0.05$). However, the association between the serum concentrations of AC/FC and the activities of SGOT ($r=0.095$, $p>0.05$) and SGPT ($r=0.283$, $p>0.05$) was not significant.

Table 4 shows comparison between the demographic, clinical and laboratory characteristics of epileptic children with abnormal carnitine levels in relation to presence or absence of hyperammonemia.

After treatment of children with abnormal ammonia and carnitine levels with L-carnitine, normalization of levels of ammonia and carnitines and liver enzymes occurred (Table 5).

Discussion

The results of this study indicate that: (1) children with primary epilepsy and treated with VPA are at risk to develop asymptomatic hyperammonemia (without encephalopathy or manifestations of hepatic failure) (20%), (2) children with primary epilepsy and treated with VPA are at risk to develop carnitine deficiency (63.4%) even in absence of neurologic disability or nutritional deficiency, (3) carnitine deficiency is a risk for hyperammonemia in patients treated with VPA as evidenced by occurrence of the latter in 33.6% of patients with carnitine deficiency and further confirmed by reversal with carnitine supplementation. The younger the age, the more the risk for development of hyperammonemia, (4) hyperammonemia is associated with elevated liver enzymes, and (5) in general, hyperammonemia secondary to VPA is of good prognosis as normalization occur with L-carnitine supplementation.

In the present study, approximately one-third of children with VPA had elevated liver enzymes and all children with hyperammonemia had elevated liver enzymes which may be considered as an indication of early liver damage. It is known that elevated liver enzymes with and without hyperammonemia, Reye syndrome or Reye-like hep-

Table 2 Concentrations of carnitine, ammonia and liver function in children with epilepsy before and after treatment with valproate.

Data	Group 1 (before treatment with VPA) ($n = 60$)	Group 2 (1 year after VPA therapy) ($n = 60$)	Control group ($n = 40$)
Total bilirubin			
Range (mg/dL)	0.20—1.50	0.20—1.50	0.20—1.10
Mean ± SD	0.58 ± 0.15	0.43 ± 0.20	0.63 ± 0.29
p-Value	$p > 0.05$[a]	$p > 0.05$[a] $p > 0.05$[b]	
SGOT			
Number (%)	0	22 (36.7%)	
Range (IU/L)	22.0—40.0	20.0—40.0	20.0—35.0
Mean ± SD	22.50 ± 2.50	37.50 ± 5.50	25.70 ± 5.72
p-Value	$p > 0.05$[a]	$p < 0.05$[a] $p < 0.05$[b]	
SGPT			
Number (%)	0	22 (36.7%)	
Range (IU/L)	4.00—35.00	4.00—35.00	4.00—37.00
Mean ± SD	9.50 ± 4. 10	12.50 ± 2.10	9.50 ± 3. 10
p-Value	$p > 0.05$[a]	$p < 0.05$[a] $p < 0.05$[b]	
Ammonia			
Number (%)		12 (20%)	
Range (μg/dL)	34.00—50.00	34.00—90.00	0.05—48.00
Mean ± SD	40.73 ± 5.42	75.56 ± 17.99	36.43 ± 10.81
p-Value	$p > 0.05$[a]	$p < 0.05$[a] $p < 0.05$[b]	
Total carnitine			
Number (%)		7 (11.7%)	
Range (μmol/L)	40.00—50.00	20.00—50.00	40.00—52.00
Mean ± SD	42.20 ± 3.17	43.27 ± 8.24	47.93 ± 3.71
p-Value	$p > 0.05$[a]	$p > 0.05$[a] $p > 0.05$[b]	
Free carnitine			
Number (%)		18 (30%)	
Range (μmol/L)	32.00—45.00	15.00—45.00	34.00—48.00
Mean ± SD	36.87 ± 4.01	25.27 ± 8.10	40.87 ± 4.76
p-Value	$p > 0.05$[a]	$p < 0.01$[a] $p < 0.05$[b]	
Acylcarnitine			
Number (%)		13 (21.7%)	
Range (μmol/L)	5.00—10.00	3.00—12.00	4.00—13.00
Mean ± SD	7.33 ± 1.63	10.00 ± 2.41	7.07 ± 2.34
p-Value	$p > 0.05$[a]	$p < 0.05$[a] $p < 0.05$[b]	
Acylcarnitine/free carnitine			
Number (%)		18 (30%)	
Range	0.11—0.29	0.04—0.50	0.08—0.36
Mean ± SD	0.19 ± 0.06	0.38 ± 0.12	0.18 ± 0.07
p-Value	$p > 0.05$[a]	$p < 0.01$[a] $p < 0.01$[b]	

SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase.
Data are expressed as range and mean ± SD.
Number (%): number of patients with abnormal values.
[a] Significance versus control group.
[b] Significance versus untreated group.

Table 3 Concentrations of carnitine and ammonia in patient's subgroups.

Data	Patients on VPA ($n=43$)	Patients on VPA + CBZ ($n=17$)	Controls ($n=40$)
Ammonia			
Range (μg/dL)	34.00—78.00	60.00—90.00	0.05—48.00
Mean ± SD	73.20 ± 5.63	53.73 ± 6.12	36.43 ± 10.81
p-Value	$p<0.05$[a]	$p<0.01$[a] $p<0.05$[b]	
Total carnitine:			
Range (μmol/L)	30.00—50.00	20.00—33.00	40.00—52.00
Mean ± SD	36.27 ± 3.84	42.33 ± 5.86	47.93 ± 3.71
p-Value	$p>0.05$[a]	$p>0.05$[a] $p>0.05$[b]	
Free carnitine:			
Range (μmol/L)	22.00—33.00	15.00—29.00	34.00—48.00
Mean ± SD	22.20 ± 3.12	29.73 ± 3.92	40.87 ± 4.76
p-Value	$p<0.001$[a]	$p<0.001$[a] $p<0.05$[b]	
Acylcarnitine:			
Range (μmol/L)	4.00—12.00	3.00—12.00	4.00—13.00
Mean ± SD	10.07 ± 3.49	9.60 ± 2.75	6.07 ± 2.34
p-Value	$p<0.05$[a]	$p<0.05$[a] $p>0.05$[b]	
Acylcarnitine/free carnitine:			
Range (μmol/L)	0.04—0.46	0.12—0.50	0.08—0.36
Mean ± SD	0.45 ± 0.12	0.31 ± 0.15	0.18 ± 0.07
p-Value	$p<0.001$[a]	$p<0.01$[a] $p<0.05$[b]	

VPA: valproate, CBZ: carbamazepine.
[a] Significance versus control.
[b] Significance versus VPA monotherapy.

atic failure may occur in epileptic patients during VPA therapy (Zaret et al., 1982; Gidal et al., 1997; Bohan et al., 2001). Inhibition of β-oxidation, and toxicity from VPA metabolites have been hypothesized for the pathogenesis of VPA-induced hepatotoxicity (Lheureux and Hantson, 2009).

Neurologists, pediatricians, internists and psychiatrists have to be aware about the increasing risk of hyperammonemia (with and without manifestations of hepatic injury) in patients with VPA. Our study is in accordance with the findings in the series of Laub (1986), Altunbasak et al. (1997) and Verrotti et al. (1999) who found that 20—50% of their series had slightly elevated plasma ammonia levels without clinical abnormalities or signs of hepatic failure. However, it has to be remembered that although rare, symptomatic hyperammonemia or hyperammonemic encephalopathy is a serious medical condition and seems to be more frequent in patients with congenital urea cycle enzymatic defects or carnitine deficiency (Rudkin and Arnold, 1999). Symptoms of VPA-induced hyperammonemic encephalopathy appeared

Table 4 Comparison between the demographic, clinical and laboratory characteristics of epileptic children with abnormal carnitine levels in relation to presence or absence of hyperammonemia.

Demographic data	Patients with hyperammonemia ($n=12$)	Patients without hyperammonemia ($n=26$)	*p*-Value
Patients' age; years	9.5 ± 2.8	13.5 ± 2.4	$p<0.05$
Male/female	7/5	10/16	$p<0.05$
Age at onset; years	4.3 ± 0.5	6.7 ± 0.8	$p<0.05$
Duration of illness; months	6.2 ± 0.5	4.5 ± 1.5	$p<0.05$
SGOT (IU/L)	75.5 ± 2.5	32.1 ± 0.4	$p<0.01$
SGPT (IU/L)	28.0 ± 0.5	12.4 ± 0.7	$p<0.05$
Acylcarnitine/free carnitine (μmol/L)	0.75 ± 0.5	0.35 ± 0.1	$p<0.01$

SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase
Data are expressed as range and mean ± SD.

Table 5 The beneficial effect of L-carnitine supplementation on liver enzymes, ammonia and carnitine levels of epileptic children.

Data	Mean values (before carnitine supplementation) ($n = 38$)	Mean values (after carnitine supplementation) ($n = 38$)	p-Value
SGOT (IU/L)	55.50 ± 2.50	27.35 ± 1.20	$p < 0.01$
SGPT (IU/L)	25.45 ± 2.35	11.50 ± 0.65	$p < 0.05$
Ammonia (μg/dL)	73.20 ± 5.07	42.06 ± 0.50	$p < 0.01$
Total carnitine (μmol/L)	32.05 ± 4.35	47.32 ± 2.25	$p < 0.05$
Free carnitine (μmol/L)	20.45 ± 6.05	40.60 ± 4.35	$p < 0.01$
Acylcarnitine (μmol/L)	15.03 ± 2.50	6.97 ± 0.53	$p < 0.05$
Acylcarnitine/free carnitine (μmol/L)	0.75 ± 0.25	0.35 ± 0.45	$p < 0.001$

SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase.
Data are expressed as range and mean ± SD.

either a few days after initiation of VPA therapy, or after several months or years (Dealberto, 2007).

The pathogenesis of hyperammonemic encephalopathy is still unclear, but it has been suggested that hyperammonemia can produce encephalopathy via inhibition of glutamate uptake by astrocytes. The elevated glutamine increases intracellular osmolarity, promoting an influx of water with resultant astrocytic swelling, compromise of astrocyte energy metabolism which results in cerebral edema with increased intracranial pressure (Wong et al., 2007; Chou et al., 2008). Panda and Radhakrishnan (2004) suggested that VPA-induced hyperammonemic encephalopathy may occur in people with normal liver function and despite normal doses and serum levels of VPA. Fortunately, it has a good prognosis, with the early withdrawal of VPA and treatment with L-carnitine, particularly in presence of normal transaminases and normal coagulation (Gerstner et al., 2006; Wadzinski et al., 2007).

The exact pathophysiological mechanisms behind hyperammonemia during VPA therapy are not completely understood. The blood ammonia level is believed to be an indicator of mitochondrial dysfunction caused by carnitine deficiency. In the present study, altered carnitine homeostasis was reported in 63.4% of children on VPA in which nearly half of them had hyperammonemia. Altered carnitine homeostasis occurred in the form of reduced total carnitine (TC), reduced free carnitine (FC), elevated acylcarnitine (AC) or acylcarnitine-to-free carnitine ratio (AC/FC). Ammonia levels were significantly correlated with carnitine levels and ratios. AC/FC ratio is considered as a highly sensitive indicator to intramitochondrial metabolic alterations (Atar et al., 1997). However, none of the epileptic children in this study had manifest hypocarnitinemia, i.e. no apparent manifestations for carnitine deficiency which include muscle weakness, hypotonia, vomiting, recurrent infection, poor appetite and poor concentration (Beghi et al., 1990; Shapira and Gutman, 1991), which are difficult to evaluate objectively in children.

Controversial data regarding carnitine status were reported among patients with primary idiopathic epilepsy on VPA therapy that are otherwise free of neurologic or nutritional problems (Hirose et al., 1998; Verrotti et al., 1999; Fung et al., 2003). This difference was attributed to different patients' selection, limited sample size and different methodologies. As patients included in this study were free of neurological complications or obvious nutritional problems, our results contradict the notion that carnitine deficiency does not occur in healthy epileptic children (Hirose et al., 1998). In accordance, carnitine deficiency was reported in 4—76% of children and adults on long-term VPA therapy (Fung et al., 2003). Hyperammonemia has been though to be a typical manifestation of secondary carnitine deficiency such as in organic acidemia (Di Donato et al., 1984). This is supported by the finding of this and other studies in which decrease in ammonia levels and an early favorable clinical response due to the probable carnitine deficiency induced in VPA-treated patients occur with L-carnitine supplementations (Ohtani et al., 1982; König et al., 1994; Gidal et al., 1997).

In the present study, approximately two-thirds of patients with abnormal carnitine levels did not have hyperammonemia. Comparing the demographic, clinical and laboratory characteristics of patients with and without hyperammonemia revealed that patients with hyperammonemia were younger, had earlier age at onset and prolonged duration of illness before starting VPA treatment and had higher levels of liver enzymes, which raises concern to consider these confounding variables as risk for hyperammonemia among children treated with VPA. In support, significant correlation has been identified between carnitine and ammonia levels, patients' age and dose of VPA (Sugimoto et al., 1987; Murakami et al., 1990; Chung et al., 1997). In contrast, some studies did not find association between the daily dose of VPA and carnitine levels (Beghi et al., 1990; Fung et al., 2003). It is important to know that infants and children are more susceptible to the adverse effects of VPA compared to adults. The neurologists must be familiar with the specific changes of AED metabolism with age. In general, metabolic rates are fastest in children; therefore, AED half-lives are shortest in this group. Rates of AED elimination are slowest in neonates, infants, and children. Thus, children need larger dosages, on mg/kg basis (three to five times higher than adults). Profiles of metabolites may also be age-specific. The relative amount of VPA metabolized to 4-ene is more than twofold less in adults than in children, which may explain the different profile of hepatotoxicity seen by age (Leppik, 1992).

The possible mechanisms for carnitine deficiency induced by VPA and the subsequent development of hyperammonemia are complex and not completely understood. Several

mechanisms have been hypothesized: (1) VPA inhibits the biosynthesis of carnitine indirectly through decreasing the level of alpha-ketoglutarate, a cofactor of butyrobetaine hydroxylase, in the liver. Butyrobetaine is a precursor of carnitine (Farkas et al., 1996), (2) VPA is toxic to liver mitochondria. It decreases intracellular adenosine triphosphate due to inhibition of β-oxidation, pyruvate metabolism, oxidative phosphorylation, gluconeogenesis and reduction of the efficiency of energy-dependent carnitine transporter (Haas et al., 1981; Coude et al., 1983; Becker and Harris, 1983; Rogiers et al., 1985), (3) VPA, carnitine, and coenzyme A (CoA) form poorly productive intracellular complexes, valproyl-CoA and valproylcarnitine. Sequential formation of valproyl-CoA and valproylcarnitine leads to direct competitive inhibition of carnitine uptake at the transporter site as follows: (a) VPA causes competition between FC and ACs, including valproylcarnitine esters and short chain acylcarnitines at plasmalemmal high affinity carnitine transporter site. Because cellular energy demands remain constant utilization of fatty acids for energy needs, inhibition of β-oxidation of fatty acid caused by VPA and results in an increase in compensatory amino acid oxidation and a subsequent increase in the production of nitrogenous waste (Millington et al., 1985) and (b) valproyl-CoA (an esterified valproic acid) required for the formation of N-acetylglutamate, a powerful allosteric activator of carbamoyl phosphate synthetase I and critical to the short-term regulation of the synthesis of urea and nitrogen flux toward its appropriate metabolism. Therefore, a VPA-induced decrease in N-acetyl glutamate could reduce the synthesis of urea and produce a rise in ammonia (Coude et al., 1983; Laub, 1986), (4) VPA also causes impairment of renal absorption of carnitine (Warter et al., 1983). Valproylcarnitine esters enhance the renal excretion of carnitine (Chalmers et al., 1984), (5) insufficient dietary intake should not be excluded as a cause of hypocarnitinemia with VPA (Borum and Bennett, 1986; Morita et al., 1986). Although, no apparent nutritional problems were identified in our patients, however, all of the above factors are compounded when treated population generally suffer from poor nutrition and chronic illness, and (6) it is also important to remember that VPA may unmask an underlying metabolic disorders associated with hepatodegeneration, mitochondrial disease or inborn errors of metabolism, e.g. heterozygous deficiency of ornithine transcarbamoylase, an important enzyme in the urea cycle. The latter is an x-linked disorder occurs in approximately 1 of 30,000 women. Laboratory findings suggestive of ornithine transcarbamoylase deficiency include elevated urine levels of orotic acid; elevated blood levels of ammonia, glutamine, and alanine; and low levels of citrulline. However, it has been found that most cases of VPA-induced hyperammonemia occur in people without this known enzyme deficiency (Oechsner et al., 1998).

In this study, patients treated with CBZ in addition to VPA had more favorable ammonia and carnitine values compared to patients on VPA monotherapy. This has been attributed to the fact that CBZ is an enzyme-inducer AED which reduce the level of VPA. This contradicts the previous observations which indicated that combination with enzyme-inducer AEDs (as phenobarbital or phenytoin) appeared to be a risk factor for carnitine deficiency. The exact underlying mechanism is unknown however, combination with other AEDs is known to enhance VPA metabolism resulting in insufficient endogenous carnitine synthesis, increased renal loss of carnitine and the production of VPA toxic metabolites (Opala et al., 1991; Verrotti et al., 1999).

Considerations for carnitine supplementation in this group of patients with early hyperammonemia and hypocarnitinemia should be discussed. In November 1996, a Pediatric Neurology Advisory Committee updated the consensus statement issued in 1989 by a panel of neurologists and metabolic experts on L-carnitine supplementation in childhood epilepsy (De Vivo et al., 1998). It has been suggested that L-carnitine supplementation should also be administered in infants and young children on VPA therapy and for older children on VPA therapy in presence of symptoms suggesting carnitine deficiency, symptomatic hyperammonemia, presence of multiple risks for hepatotoxicity and persistent hypocarnitinemia when free carnitine levels is checked at periodic basis (Ohtani et al., 1982). König et al. (1994) suggested that carnitine supplementation in patients on VPA is necessary as serious conditions as hepatotoxicity may be sudden and unpredictable. Carnitine is safe and well tolerated drug, however, considerations regarding assessment of the dosage of carnitine is needed. The recommended dose in secondary carnitine deficiency has been suggested to be 50—100 mg/(kg day) and dose titration depends on the restoration of carnitine stores (De Vivo et al., 1998). However, carnitine supplementation has not been studied satisfactorily and the possible value of treatment is based on understanding the pathophysiologic processes associated with carnitine deficiency. The potential benefits of L-carnitine supplementation include: (a) maintenance of mitochondrial metabolism, (b) decrease in impairment of plasmalemmal free carnitine uptake into tissue through an increase in free carnitine concentration at transporter site, (c) increased free carnitine would also provide a greater buffering capacity for excessive potentially toxic acyl-CoAs, including valproyl-CoA, thereby decreasing secondary inhibition of fatty acid oxidation, pyruvate oxidation, and gluconeogenesis and increasing intramitochondrial free CoA (Verrotti et al., 1999), and (d) it is postulated that carnitine supplementation may increase the β-oxidation of VPA, thereby limiting cytosolic omega-oxidation and the production of toxic metabolites that are involved in liver toxicity and ammonia accumulation (Lheureux and Hantson, 2009).

Finally, despite the strengths of our study, some limitations are raised: (1) the included control subjects were not 100% healthy, although apparently they had minor illness (e.g. fever or sore throat), it has to be remembered that some infectious diseases can also influence metabolism, (2) some patients were on polytherapy which may affect the interpretation of the results. Further studies are needed to evaluate the effect of different combination with enzyme-inducer and non-enzyme-inducer AEDs on the metabolism of VPA, (3) food diaries for patients and control subjects were not meticulously obtained to check for the presence of nutritional problems that may affect the carnitine levels. On the other hand, some studies provide evidence that transient hyperammonemia may occur with protein overload (Gidal et al., 1997), (4) although rare, an underlying metabolic disorders associated with hepatodegeneration, mitochondrial disease or inborn errors of metabolism have

to be looked for (through determination of related amino acids) in cases of VPA-induced hyperammonemia. For example, elevated urine levels of orotic acid; elevated blood levels of ammonia, glutamine, and alanine; and low levels of citrulline are indications for heterozygous deficiency of ornithine transcarbamoylase (Oechsner et al., 1998), and (5) this study lack serial or periodic follow up of blood levels (e.g. every 1—3 months) of ammonia and carnitine as persistence of abnormalities on follow up, particularly in the high risk group, is a risk for manifest hyperammonemia and an indication of L-carnitine supplementation.

Conclusions

With the increasing indications and off-label uses of VPA, neurologists should be aware of the potential metabolic complications of VPA and periodically check ammonia levels in patients taking VPA who present with alterations in mental status. Also as carnitine deficiency occur in the course of treatment with VPA and linked to the risk of hyperammonemia, regular clinical follow up of patients is required as manifestations of carnitine deficiency are frequently suspected clinically and confirmed by lab studies. Neurologists should be aware that persistence of abnormalities, particularly in association with abnormal transaminases may be an indication for early carnitine supplementation, reducing VPA dose or consideration of alternative AED. Attention should also be paid to drug interaction with VPA which may aggravate manifestations of hyperammonemia. Also it has to be remembered that ethnicity and augmentation of the existent nutritional deficiency are among the important causes of hypocarnitinemia. Further clinical and basic researches are important to provide information about: (a) the exact risks and clinical significance of carnitine deficiency and their effect on seizure control, (b) the efficacy of prophylactic carnitine supplementation in the prevention of hepatotoxicity, and (c) the cost-effective dose required for patients with hypocarnitinemia and the effect of carnitine supplementation on VPA metabolism and seizure control.

Conflicts of interest

None.

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