Original article

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Analysis of acylcarnitine levels by tandem mass spectrometry in epileptic children receiving valproate and oxcarbazepine

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ABSTRACT – This prospective study was designed to investigate whether or not monotherapy with sodium valproate (VPA) or oxcarbazepine (OXC) affects plasma levels of fatty acylcarnitine esters in children with epilepsy. A total of 56 children with idiopathic partial or generalised epilepsy were included in the study. Patients were assigned to receive either VPA or OXC monotherapy. Free carnitine (C0) and acylcarnitine profiles of the patients were investigated using tandem mass spectrometry at baseline and at six and 18 months after commencement of therapy. For patients receiving VPA or OXC monotherapy, there were no significant differences in plasma levels of C0, compared with baseline, at six and 18 months ($p>0.05$). Treatment with VPA for six and 18 months correlated with a significant increase in 3-hydroxy-isovalerylcarnitine (C5-OH) (six months: +23%; 18 months: +73%), and significant decreases in the following acylcarnitines: C6-acylcarnitine (six months: -60%; 18 months: -66%), C14-acylcarnitine (six months: -25%; 18 months: -38%), C16-acylcarnitine (six months: -73%; 18 months: -73%), and C18:1-OH-acylcarnitine (six months: -60%; 18 months: -70%), compared with baseline ($p<0.05$). In patients receiving OXC monotherapy, on the other hand, plasma concentrations (μmol/L) of acylcarnitines (from C2 to C18:1-OH) fell within the normal reference range. The results of this study indicate that there are significant biochemical changes in acylcarnitines in ambulatory children on VPA monotherapy but these are not clinically significant. OXC monotherapy had no effect on acylcarnitine metabolism in ambulatory children.

Key words: acylcarnitine, children, epilepsy, valproate, oxcarbazepine

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The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to enzymes responsible for β-oxidation. Oxidation of

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long-chain fatty acids in the mitochondria provides an important source of energy for the heart as well as for skeletal muscle during prolonged aerobic work and for hepatic ketogenesis during long-term fasting (Stanley, 2004). Carnitine deficiency causes clinical symptoms such as gastrointestinal dysmotility, manifested by vomiting, delayed gastric emptying, and constipation, as well as other symptoms such as muscle weakness, hypotonia, lipid storage myopathy, cardiomyopathy, failure to thrive, and even encephalopathy (Kurul *et al*., 2003).

Antiepileptic drugs (AEDs) have long been known to affect carnitine metabolism (Hug *et al*., 1991; Zelnik *et al*., 1995). According to some authors, of the old-generation AEDs, the broad-spectrum antiepileptic valproate (VPA) is the strongest carnitine-reducing agent (Opala *et al*., 1991; Thom *et al*., 1991; Riva *et al*., 1993; Van Wouwe, 1995). Some authors have indicated that the observed complications are due to the combination of carnitine reduction and other factors believed to further the risk of carnitine deficiency. These risk factors include young age, polytherapy, poor nutritional status, and underlying neurological (especially neurometabolic) and liver diseases (Coulter, 1991; Coulter, 1995). However, it is controversial whether VPA constitutes a risk for "otherwise healthy" epileptic patients without any of these risk factors (Hirose *et al*., 1998; Fung *et al*., 2003; Werner *et al*., 2007; Hamed and Abdella, 2009). The effects of new-generation AEDs on plasma carnitine levels have been only recently investigated, and only in small numbers of patients (Coppola *et al*., 2006); these data are therefore considered preliminary (Zelnik *et al*., 2008). Oxcarbazepine (OXC), a new AED, is chemically and structurally similar to carbamazepine. Few studies have investigated whether or not OXC has an effect on carnitine levels (Kurul *et al*., 2003).

In the current study, we prospectively evaluated the effects of VPA and OXC monotherapy, after six and 18 months, on serum carnitine levels in children with epilepsy, and compared these serum carnitine levels to those measured at baseline.

Materials and methods

Fifty-six newly diagnosed paediatric patients (31 girls and 25 boys), ranging in age from three to 17 years, with partial or generalised epilepsy, were enrolled in this study. None of the patients were being treated with any AEDs or any other drugs that might affect acylcarnitine function. Patients did not have any endocrine, cardiac, or neurometabolic diseases or any other chronic diseases.

Additional exclusion criteria for the study included carnitine medication, liver disease (abnormal liver function test), endocrine disorder (*i.e*. diabetes mellitus), muscle disease (abnormal creatine kinase levels), cardiac disease, renal disease (abnormal renal function test), ketogenic or vegetarian diets, and metabolic disorders. Seven patients were excluded because of endocrine disorders, cardiac disease, or other chronic diseases.

Each patient was examined by the same physician at baseline, and at six and 18 months after commencement of therapy. The clinical status of each patient was recorded.

Overall, 28 patients (13 girls and 15 boys) received OXC monotherapy while the remaining 28 (18 girls and 10 boys) received VPA monotherapy.

Seizure type was classified according to the criteria established by the International League Against Epilepsy (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Either OXC or VPA treatment was initiated in patients with partial epilepsy, while VPA was used for all patients with primary generalised epilepsy. The AED dose was chosen according to generally accepted guidelines (Scheuer and Pedley, 1990). OXC and VPA were administered twice daily.

Levels of free carnitine (C0) and acylcarnitines (from C2 to C18:1-OH) were evaluated at baseline, and at six and 18 months after commencement of therapy in all patients. During the study period, six children receiving VPA therapy were excluded from the study due to the use of a second AED (two children at six months and four children at 18 months). Similarly, three children receiving OXC therapy were excluded from the study (one child at six months and two children at 18 months).

To determine levels of free carnitine and acylcarnitines in blood, blood specimens from the patients were spotted onto Guthrie cards 12 hours after the last drug dose. The specimens were then studied using tandem mass spectrometry (Tandem-MS), a method suggested by Chace *et al*. (1997). The results were given as μmol/L.

Serum concentrations of VPA were measured using the fluorescence polarisation method with an AxSym analyzer (Abbott Diagnostic Division, Irving, TX, USA). Serum concentrations of monohydroxy-carbamazepine (the active metabolite of OXC) were measured using high-performance liquid chromatography (HPLC).

The current study was carried out in accordance with the principles outlined in the Declaration of Helsinki. Consent forms were obtained from all of the patients and/or their parents. The study was approved by the local Ethics Committee of Gazi University, Faculty of Medicine.

Statistical analysis

Statistical analysis was conducted using SPSS for Windows (version 11.5; SPSS Inc., Chicago, IL, USA). Descriptive statistics are presented as mean ± standard deviation. The normality assumption was tested using analysis of variance (ANOVA). If normality was not achieved, the Friedman two-way ANOVA was used. Significance for differences (repeated measures ANOVA) associated with follow-up times between groups was determined by paired t-test. When the Friedman multiple comparison test was used and significant differences were found between follow-up times, a Wilcoxon Sign Test was applied. A p value <0.05 was considered significant.

Results

Twenty-eight (13 girls and 15 boys) patients receiving OXC monotherapy and 28 patients (18 girls and 10 boys) receiving VPA monotherapy were evaluated. The patients' characteristics are summarised in *table 1*.
Plasma concentrations (μmol/L) of free carnitine and acylcarnitines in children receiving VPA and OXC are given in *tables 2* and *3*, respectively.
In patients receiving VPA, significant differences were noted between baseline levels and levels after treatment for six and 18 months with the following acylcarnitines ($p<0.05$): C6-acylcarnitine [baseline: 0.30±0.04 μmol/L, six months: 0.12±0.10 μmol/L (-60%), 18 months: 0.10±0.11 μmol/L (-66%)], 3-hydroxy-isovalerylcarnitine (C5-OH) [baseline: 0.22±0.07 μmol/L, six months: 0.27±0.12 μmol/L (+23%), 18 months: 0.38±0.11 μmol/L (+73%)], C14-acylcarnitine [baseline: 0.16±0.07 μmol/L, six months: 0.12±0.04 μmol/L (-25%), 18 months: 0.10±0.04 μmol/L (-38%)], C16-acylcarnitine [baseline: 0.22±0.30 μmol/L, six months: 0.06±0.05 μmol/L (-73%), 18 months: 0.06±0.08 μmol/L (-73%)], and C18:1-OH-acylcarnitine [baseline: 0.10±0.16 μmol/L, six months: 0.04±0.03 μmol/L (-60%), 18 months: 0.03±0.02 μmol/L (-70%)]. Baseline levels were higher than levels after treatment for six and 18 months for all of these acylcarnitines, except C5-OH. For C0 as well as other acylcarnitine species, no significant differences were found between baseline levels and levels after treatment for six and 18 months in these patients.
Plasma concentrations (μmol/L) of free carnitine and acylcarnitines in patients receiving OXC monotherapy fell within normal reference ranges at baseline, and at six and 18 months of treatment.

Discussion

The results of the current study indicate that VPA and OXC do not have significant effects on carnitine and acylcarnitine metabolism in ambulatory epileptic children, with the exception of alterations in specific acylcarnitine species in patients receiving VPA monotherapy.
In contrast to studies reporting significant effects of VPA therapy on serum carnitine status (Riva *et al*., 1993; Van Wouwe, 1995; Castro-Gago *et al*., 1998; Werner *et al*., 2007), in the current study, levels of C0 decreased by the eighteenth month but this difference did not reach statistical significance. Similar findings were also reported in 1998 by Hirose *et al*. The authors found that the amount of carnitine that children on a regular diet ingested was far beyond their daily carnitine requirement. Neither levels of total carnitine, nor levels of C0, appeared to be affected by VPA therapy in epileptic patients without severe neurological or nutritional problems. The authors concluded that carnitine deficiency caused by VPA therapy was not likely to occur in epileptic patients without severe neurological or nutritional problems because blood carnitine level depended on nutritional condition rather than blood VPA concentration (Hirose *et al*., 1998). Fung *et al*. (2003) showed that among 43 patients receiving VPA, only two patients had carnitine levels below the normal limit. The authors found no significant association between carnitine levels and age, body mass index, additional AEDs used, presence of mental retardation, cerebral palsy, feeding problems, non-ambulatory status, or dosage of VPA. The authors also concluded that routine carnitine level checking was not justified in paediatric patients receiving VPA (Fung *et al*., 2003).
In agreement with previous studies on serum carnitine status (Silva *et al*., 2001a; Werner *et al*., 2007),

Table 1. Clinical characteristics of patients receiving antiepileptic monotherapy in the current study.

	n	Age (years)	Dosage (mg/kg)	Type of epilepsy		Drug serum concentration (μg/mL)	
				Partial	Generalised	Month 6	Month 18
Oxcarbazepine	28	8.95±3.48	20-30	28	0	18.90±5.18	21.55±6.25
Valproate	28	10.11±4.16	20-35	16	12	72.96±23.76	70.36±21.52

Table 2. Plasma concentrations (μmol/L) of free carnitine (C0) and acylcarnitines (from C2 to C18:1-OH) of patients receiving Sodium Valproate monotherapy.

Valproate (*n*=28)	Patients		
	Baseline	Month 6	Month 18
*C0	32.86±10.89	32.55±10.84	29.57±7.1
*C2	27.85±7.65	26.42±8.53	25.78±9.8
*C3	2.38±1.42	2.52±1.13	1.98±0.87
**C4	0.45±0.22	0.43±0.40	0.42±0.25
*C5:1	0.06±0.03	0.05±0.03	0.05±0.03
*C5	0.19±0.09	0.18±0.07	0.17±0.07
*Butyrylcarnitine	0.11±0.04	0.09±0.03	0.09±0.03
**C6¶	0.30±0.04	0.12±0.10[a]	0.10±0.11[a]
*C5-OH#	0.22±0.07	0.27±0.12[c]	0.38±0.11[c]
*C8	0.11±0.05	0.12±0.04	0.12±0.06
*C10	0.15±0.12	0.14±0.07	0.13±0.06
**Glutarylcarnitine	0.06±0.02	0.07±0.04	0.08±0.06
*C12	0.13±0.08	0.15±0.07	0.11±0.07
*Methyl-glutarylcarnitine	0.05±0.03	0.06±0.03	0.06±0.03
*C14:1	0.10±0.07	0.09±0.04	0.09±0.07
*C14#	0.16±0.07	0.12±0.04[b]	0.10±0.04[c]
*C14-OH	0.04±0.02	0.03±0.02	0.03±0.02
**C16¶	0.22±0.3	0.06±0.05[c]	0.06±0.08[c]
*C16:1-OH	0.10±0.04	0.08±0.04	0.09±0.06
*C16-OH	0.05±0.02	0.05±0.04	0.04±0.04
*C18:1	0.96±0.30	0.89±0.29	0.82±0.28
**C18:1-OH¶	0.10±0.16	0.04±0.03[b]	0.03±0.02[c]

C*n*: acyl residues with n carbons; C*n*:1: acyl residues with n carbons and monounsaturated. Data are presented as mean±SD; *Repeated measures analysis of variance; **Friedman two way analysis of variance; ¶Wilcoxon Sign test; #paired t test; [a]$p<0.05$ compared with baseline; [b]$p<0.01$ compared with baseline; [c]$p<0.001$ compared with baseline.

we observed a decrease in C2. However, this difference did not reach statistical significance, in contrast to other studies on serum carnitine status (Silva *et al*., 2001a; Werner *et al*., 2007). Reduced formation of C2 by suppressed mitochondrial β-oxidation of fatty acids can be caused by the impairment of mitochondrial acyl-coenzyme A (CoA) dehydrogenases, which catalyse the first step of the mitochondrial β-oxidation loop (Werner *et al*., 2007). In addition to the inhibition of mitochondrial fatty acid ß-oxidation and toxicity from VPA metabolites, the impairment of oxidative phosphorylation is also a highly important mechanism leading to mitochondrial dysfunction and hepatotoxicity in VPA-induced damage (Haas *et al*., 1981; Rumbach *et al*., 1983; Luis *et al*., 2007; Aires *et al*., 2008; Lheureux and Hantson, 2009). Oxidative phosphorylation is regulated by a complex variety of factors (such as genetic and metabolic factors), associated with pathways that may be inhibited by VPA which may compromise normal mitochondrial function (Luis *et al*., 2007; Silva *et al*., 2008; McFarland *et al*., 2009). As a surrogate of mitochondrial functions,

Table 3. Plasma concentrations (μmol/L) of free carnitine (C0) and acylcarnitines (from C2 to C18:1-OH) of patients receiving Oxcarbazepine monotherapy.

Oxcarbazepine (*n*=28)	Patients		
	Baseline	Month 6	Month 18
*C0	31.52±9.75	32.80±11.56	30.87±10.09
*C2	29.81±9.25	27.24±8.9	30.28±13.52
*C3	2.62±1.12	2.67±1.34	2.58±1.42
*C4	0.39±0.23	0.43±0.2	0.45±0.42
*C5:1	0.06±0.03	0.05±0.03	0.05±0.03
*C5	0.22±0.10	0.21±0.08	0.18±0.07
**Butyrylcarnitine	0.12±0.13	0.11±0.06	0.11±0.09
**C6	0.32±0.36	0.32±0.10	0.31±0.56
**C5-OH	0.24±0.09	0.23±0.10	0.24±0.11
**C8	0.10±0.06	0.11±0.06	0.12±0.06
*C10	0.14±0.11	0.15±0.10	0.17±0.11
*Glutarylcarnitine	0.08±0.05	0.09±0.09	0.08±0.04
*C12	0.14±0.09	0.13±0.07	0.15±0.07
*Methyl-glutarylcarnitine	0.06±0.04	0.07±0.04	0.07±0.03
*C14:1	0.08±0.04	0.07±0.05	0.08±0.03
*C14	0.12±0.08	0.11±0.07	0.10±0.05
*C14-OH	0.03±0.01	0.04±0.02	0.03±0.02
**C16	0.23±0.17	0.20±0.11	0.20±0.11
*C16:1-OH	0.08±0.04	0.10±0.06	0.09±0.04
*C16-OH	0.05±0.03	0.06±0.05	0.06±0.02
*C18:1	1.10±0.33	1.07±0.58	1.00±0.32
*C18:1-OH	0.09±0.04	0.08±0.04	0.09±0.03

C*n*: acyl residues with n carbons; C*n*:1: acyl residues with n carbons and monounsaturated; Data are presented as mean±SD; *Repeated measures analysis of variance; **Friedman two way analysis of variance.

urinary organic acid profiles vary particularly in young children. Price *et al*. (2011) recently reported that the organic acids, pimelic, 2-hydroxyglutaric, 4-hydroxyphenylpyruvic, succinic, glycolic, azelaic and 3-methylglutaric acids increase in patients using VPA. Except for 4-hydroxyphenylpyruvic, these organic acids are dicarboxylic acids known to be excreted in greater quantities under conditions of oxidative stress and impaired fatty acid oxidation (Price *et al*., 2011).

In agreement with Silva *et al*. (2001a) and Werner *et al*. (2007), we found increased C5-OH after treatment with VPA for six and 18 months. The increase in C5-OH was consistent with the increase in 3-hydroxy-isovalerylcarnitine level and 2-methyl-3-hydroxy-glutarylcarnitine level in patients receiving VPA. This finding might suggest an interactive mechanism between VPA and leucine metabolism; namely, an inhibition by VPA or any of its metabolites of 3-methylcrotonyl-CoA carboxylase (Silva *et al*., 2001b). Medium-chain acyl-CoA dehydrogenase deficiency, the most common metabolic disease causing Reye-like liver failure, is characterised by increased C8 and

decreased C2 (Ziadeh *et al*., 1995; Saudubray *et al*., 1999). In contrast to the findings of Werner *et al*. (2007), we found neither significantly decreased C2 nor increased C8 in ambulatory children receiving VPA therapy. Nevertheless, the finding of decreased C2, although not significant, is of importance since this finding indicates that physicians should be particularly careful when prescribing AEDs to young paediatric patients who are at high risk of hepatotoxicity and who have poor nutritional status. In agreement with Werner *et al*. (2007), we found a decrease in long-chain acylcarnitines with VPA monotherapy. However, this decrease reached statistical significance only for C18:1-OH concentrations. Werner *et al*. (2007) presented evidence for changes of acylcarnitine species that were associated with VPA therapy in epileptic children. The treatment interval with the most marked changes coincided with the interval of highest risk for VPA-induced hepatotoxicity. In this particular study, no significant changes in acylcarnitine species were observed in children receiving carbamazepine monotherapy, as compared with the control group (Werner *et al*., 2007). Silva *et al*., 2001a reported that the ratios of acylcarnitine/carnitine or long chain acylcarnitine/carnitine correlated in a positive manner with VPA concentration, suggesting that in humans, high concentrations of VPA in the plasma can lead to an imbalance in these ratios. Accordingly, the authors suggested that physicians should be careful, especially with regards to inhibition of mitochondrial β-oxidation of fatty acids in children receiving VPA monotherapy, and in particular, for patients with high ammonia levels, which may be evidence of the inhibition of the mitochondrial fatty acid β-oxidation pathway (Rumbach *et al*., 1983). However, elevated plasma ammonia can be observed even in patients with normal liver tests, and seems therefore to result from underlying mechanisms that are independent of hepatotoxicity (Lheureux and Hantson, 2009). Hyperammonaemia in patients under VPA treatment may also result from a direct effect of VPA on the mitochondrial urea cycle enzyme CPS I as well as an indirect effect on CPS I by direct inhibition of N-acetylglutamate (NAGS) activity (Rumbach *et al*., 1983).

Previous studies have suggested that carbamazepine monotherapy causes carnitine deficiency in children with primary idiopathic epilepsy (Hug *et al*., 1991; Castro-Gago *et al*., 1998; Kurul *et al*., 2003). Kurul *et al*. (2003) measured serum concentrations of total carnitine and C0 in 20 otherwise healthy children with primary idiopathic epilepsy who were receiving OXC monotherapy, and found no significant difference between serum concentrations of total carnitine and C0 at the third and sixth months of therapy. Similarly, we found that OXC monotherapy had no significant effect on carnitine and acylcarnitine metabolism, during an 18-month period, in otherwise healthy children with primary generalised epilepsy treated with OXC monotherapy.

The data obtained from our evaluation indicate that VPA, despite significant biochemical changes in acylcarnitines in ambulatory children, and OXC do not clinically significantly affect carnitine and acylcarnitine metabolism. Although carnitine deficiency is not uncommon among patients receiving VPA, it seems that in most cases carnitine deficiency depends on other parameters, such as dosage and underlying diseases. Based on these observations, we do not consider it necessary to routinely monitor carnitine levels in epileptic ambulatory children receiving VPA and OXC. Exceptions would include symptomatic cases, young infants, patients with underlying metabolic disorders, or children with liver toxicity or encephalopathy. □

Disclosure.
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