Alterations in the Carnitine Metabolism in Epileptic Children Treated with Valproic Acid

Serum concentrations of total carnitine, free carnitine and acylcarnitine were measured in forty-one epileptic patients treated with valproic acid (VPA). Among them, 14 patients were on VPA monotherapy and 27 were on VPA polytherapy. Forty-one age and sex matched healthy normal controls were also evaluated for carnitine metabolism. The mean total and free carnitine were significantly lower in both the VPA monotherapy and polytherapy groups compared with the controls. However, there were no significant differences in concentrations of carnitine between the VPA polytherapy and VPA monotherapy groups. Patients treated with VPA polytherapy had lower carnitine than those treated with VPA monotherapy. An inverse correlation was found between serum concentrations of carnitine and duration of treatment in patients treated with VPA. However, there was no significant correlations between serum concentrations of carnitine and those of VPA. Also, correlation between serum concentrations of carnitine and the activities of serum GOT and GPT was not significant. After L-carnitine supplementation in eleven patients with hypocarnitinemia, the concentrations of carnitine were significantly increased.
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Key Words : *Carnitine; Valproic acid; Aminotransferases; Carnitine acyltransferases*

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INTRODUCTION

Valproic acid (2-n-propylpentanoic acid, VPA) is one of the widely used antiepileptic drugs (AEDs) in the treatment of seizures peculiar to infants and children. VPA is relatively safe in terms of side effects, however, patients treated with VPA have been observed to experience a variety of adverse effects, such as hyperammonemia, lethargy, unexplained stupor, and a Reye-like syndrome (1-3). The most serious problem in patients treated with VPA is a Reye-like syndrome of hepatic encephalopathy (4-6).

A reduction in serum concentrations of carnitine related to VPA treatment has been repeatedly reported (4-9). Carnitine acts as a cofactor in the transfer of fatty acid across the inner membrane of mitochondria, resulting in energy production via beta-oxidation (10, 11). There are some important observations in previous reports related to VPA and alteration of carnitine. First, VPA, a strong inhibitor of fatty acid, reduces beta-oxidation leading to secondary carnitine deficiency (12-14). Second, VPA is toxic to isolated liver mitochondria (15). Third, VPA is a substrate for acylcarnitine transferase and conjugation, and excretion of valproylcarnitine may produce carnitine deficiency (16). Serum concentrations of carnitine have been found to be lower in VPA treated patients, therefore, there has been speculation as to the possible role of the lowered carnitine levels in the etiology of VPA-induced hepatopathy (7, 17, 18).

This study examined the influence of VPA on serum carnitine concentrations among patients treated with VPA monotherapy, VPA polytherapy, and controls. We tried to correlate carnitine concentrations with other parameters such as duration of treatment, s-GOT, GPT, and serum concentrations of VPA in patients with VPA treatment. In addition, we investigated the effect of L-carnitine supplementation in patients showing lower carnitine concentrations by measuring the serum concentrations of carnitine longitudinally, in the same patients before and after supplementation.

MATERIALS AND METHODS

Subjects

Forty-one VPA treated epileptic patients (male; 28, female; 13) and 41 age and sex matched control sub-

Table 1. Clinical summaries of valproic acid treated patients

	Group 1 (1a/1b)	Group 1c
No. of patients	47 (14/27)	11
Age (average)	8 years 7 months	10 years 8 months
Sex (No. patients)		
male	28 (13/15)	7
female	13 (1/12)	4
Seizure types (No. patients)		
West syndrome	4 (0/4)	1
LGS	9 (2/7)	3
GTC	7 (6/1)	0
Atonic	2 (0/2)	0
CPS	15 (5/10)	6
SPS	4 (1/3)	1
Levels of AEDs (μg/ml)		
Valproic acid	55.7±30.5*	56.7±24.5*
carbamazepine	6.7±2.3*	5.6±3.2*
phenytoin	3.5±1.4*	3.1±1.2*
Phenobarbital	21.3±5.6*	20.3±4.7*
Transaminase (IU)		
GOT	25.7±2.5*	25.3±3.4*
GPT	8.6±2.3*	9.5±3.1*

* Mean±SD
LGS : Lennox-Gastaut syndrome, GTC : generalized tonic clonic
CPS : complex partial seizure, SPC : simple partial seizure
AEDs : antiepileptic drugs

jects were selected for the study. The relevant clinical summaries of the VPA treated patients are given in Table 1.

They were classified as group 1, and were further subdivided into 3 groups; 14 patients treated with VPA monotherapy as group 1a, 27 patients treated with VPA polytherapy (VPA plus other antiepileptic drugs) as group 1b, and 11 patients with marked decreases in total and free carnitine concentrations receiving L-carnitine (Sigma Tau, Rome, Italy) therapy as group 1c.

Methods

We measured the serum total carnitine, free carnitine and acylcarnitine concentrations in the VPA treated patients and the controls. Each group was compared with the control. We assessed the correlation between the serum concentrations of carnitine and some parameters such as duration of drug administration, blood level of VPA, and serum transaminase (GOT/GPT) in VPA treated patients.

After supplementation of L-carnitine (50 mg/kg/day) for an average of 5.1 months (range: 4 to 7 months), the concentrations of serum total, free carnitine and acylcarnitine were measured in 11 patients showing marked hypocarnitinemia longitudinally.

Serum total and free carnitine concentrations were measured in a TBA-80PR (Toshiba Co., Japan) automatic analyzer by enzyme cycling method, and acylcarnitine concentration was calculated by subtracting free carnitine from total carnitine concentrations. The blood levels of antiepileptic drugs (AEDs) were determined with a TDX analyzer (Abbott Lab. Diagnostic Division Co., U.S.A) by fluorescence polarization immunoassay (FPIA), and the activities of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured using an automatic analyzer in all individuals. The results of serum concentrations of AEDs and GOT/GPT are presented in Table 1. Paired t-test and simple linear regression were used for statistical evaluation using StatView II.

RESULTS

The mean total carnitine and free carnitine concentrations were significantly lower in Group 1 including both the VPA monotherapy (1a) and polytherapy (1b) groups as compared to the controls ($P<0.005$) (Table 2). There were no significant differences between group 1a and 1b, however, serum concentrations of total and free carnitines in group 1b were lower than those of group 1a ($P>0.05$) (Table 2).

There was a significant inverse correlation between the total and free carnitine concentrations and the duration of VPA administration in all patients treated with VPA (group 1) (total; $r^2=0.35$, $P<0.005$, free; $r^2= 0.42$, $P< 0.005$) (Fig. 1).

Table 2. Serum concentrations of carnitine in VPA treated patients and controls

(μmol/ml)

	Control (mean±SD)	Group 1 (mean±SD)	Group 1a (mean±SD)	Group 1b (mean±SD)
Total carnitine	77.71±5.55	57.24±14.08*	58.37±15.94**	56.73±13.57
Free carnitine	67.68±6.17	47.45±12.72*	48.52±13.18**	46.97±12.09
Acylcarnitine	10.05±1.44	9.98±0.72	10.01±2.68	9.91±2.82

* Control : Group ($P<0.005$)
** Control : Group 1a or 1b ($P<0.005$)

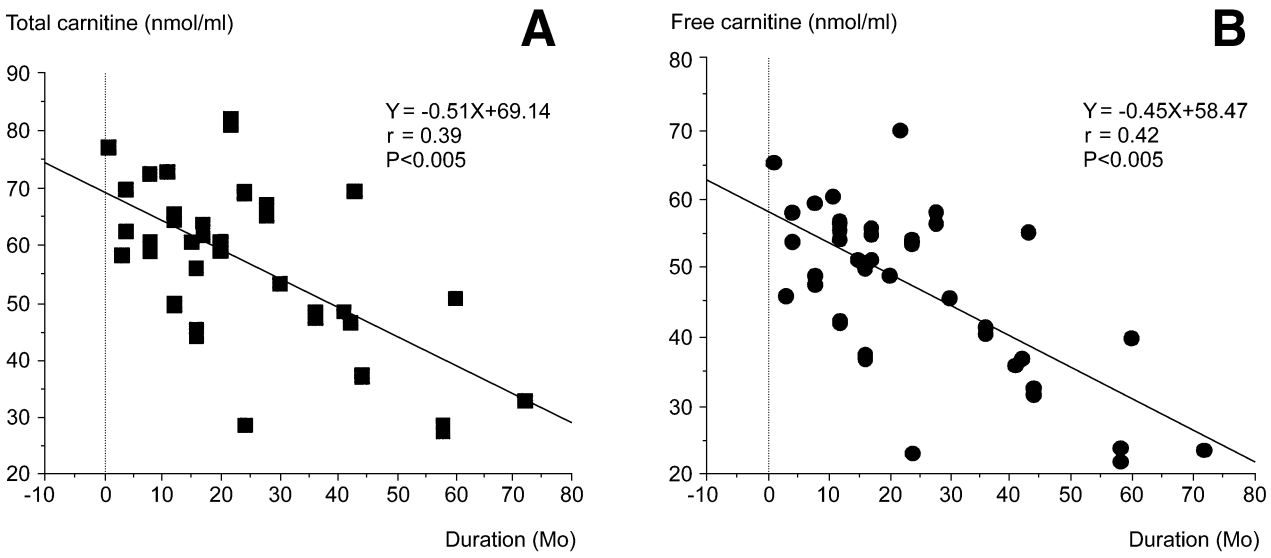

Fig. 1. Scattergrams show the correlation between serum concentrations of total carnitine (A) and free carnitine (B), and duration of VPA treatment.

There was no significant correlation between the total and free carnitine concentrations and blood levels of VPA in group 1 (total; r^2=0.09, p>0.5, free; r^2=0.09, p>0.5) (Fig. 2).

No significant correlation was observed between the concentrations of serum carnitine and the activities of serum GOT and GPT (Fig. 3) in patients treated with VPA (total; r^2=0.001, p>0.5, free; r^2=0.002, p<0.5). Within the groups Ic, 1a and 1b, there were no significant differences.

After oral supplementation of L-carnitine in 11 VPA treated patients showing marked hypocarnitinemia, serum free and total carnitine concentrations were significantly increased, and became normalized (Table 3).

DISCUSSION

The etiology of hypocarnitinemia with VPA treatment is somewhat speculative as previously described. In addition, carnitine deficiency in VPA treated patients may be due to dietary carnitine deficiency (19). Most of the

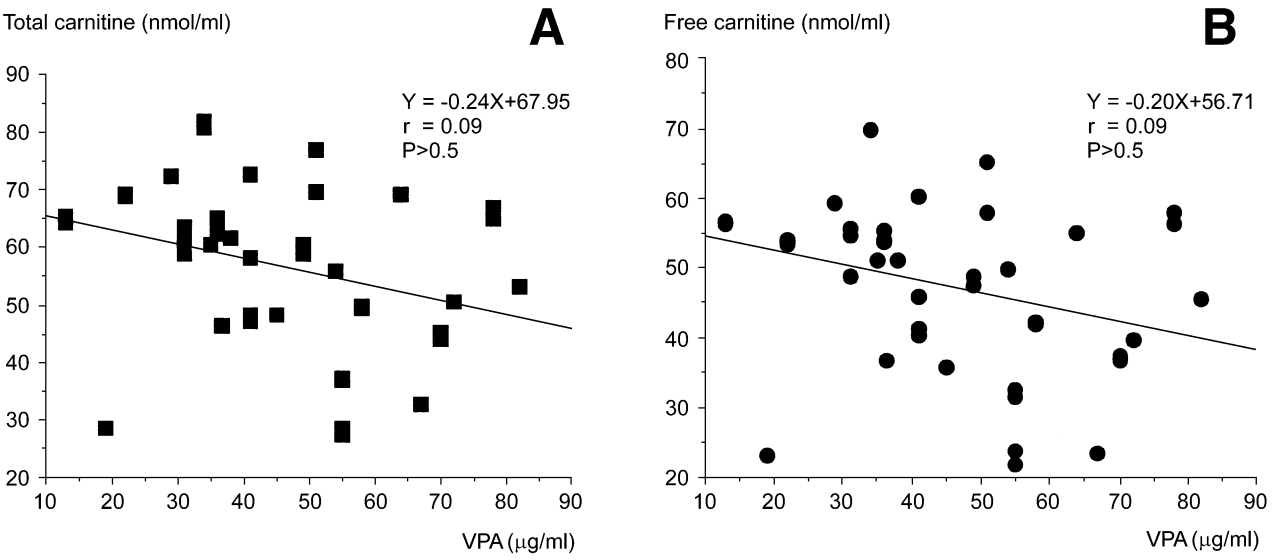

Fig. 2. Scattergrams show the correlation between serum concentrations of total carnitine (A) and free carnitine (B), and serum levels of VPA.

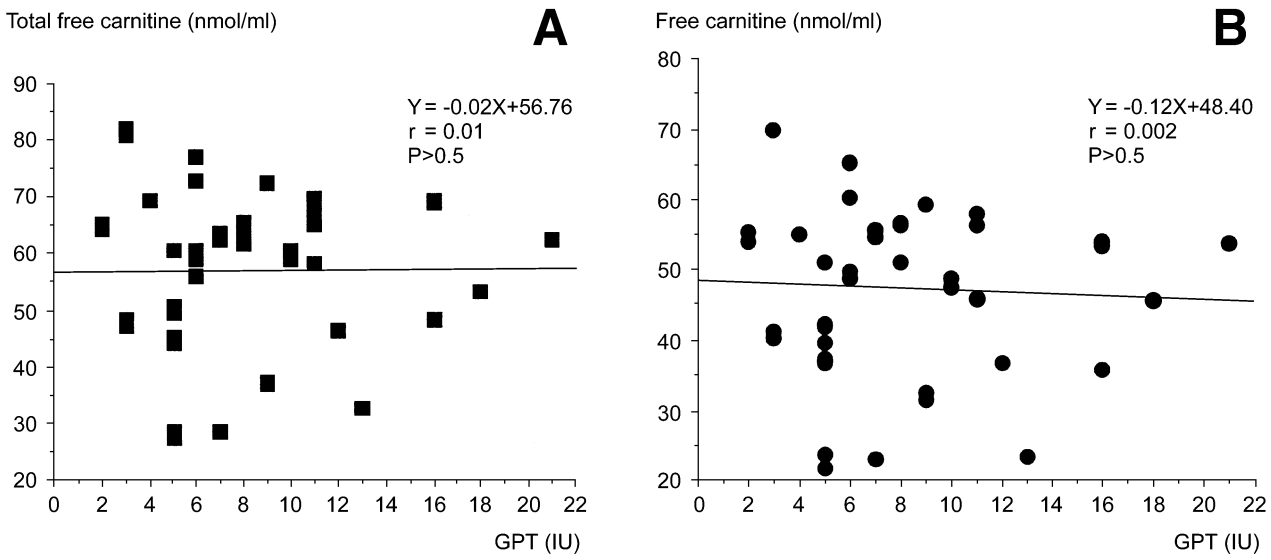

Fig. 3. Scattergrams show the correlation between serum concentrations of total carnitine (A) and free carnitine (B), and serum levels of GPT.

Table 3. Serum concentrations of carnitine before and after L-carnitine supplemenetation in group 1c

(μmol/ml)

	Before treatment (mean±SD)	After treatment (mean±SD)
Total carnitine	41.31±10.20	89.00±4.80*
Free carnitine	33.90±7.91	78.31±4.06*
Acylcarnitine	7.84±2.81	10.68±2.24

* P<0.005

reported side effects including Reye syndrome or Reye-like syndrome hepatic failure during VPA therapy have been associated with carnitine deficiency (1, 5, 14).

Our study showed significantly lower serum total carnitine and free carnitine in both the VPA monotherapy and polytherapy groups as compared to the normal controls. We found the total and free carnitine levels in the VPA polytherapy group lower than those in the VPA monotherapy group, however, there were no statistical differences. These findings supported previous studies showing lowered carnitine levels through administration of VPA (1, 5, 7, 8, 11, 20-22). They suggest that the reduction of serum carnitine levels is associated with VPA therapy, but not with other AEDs (7-9). Moreover, they also suggested that treatment with VPA in combination with other AEDs rather than with VPA monotherapy increased the reduction of carnitine levels. It is likely that drug interactions can lead to enhanced valproic acid metabolism and further impairment of carnitine levels.

In addition, Opala et al. (8) reported that comparison of VPA polytherapy and monotherapy yielded significantly lower free carnitine levels in the polytherapy group, therefore, they indicate that a general decrease in the carnitine pool should be anticipated in patients taking VPA polytherapy and, to a lesser degree, monotherapy.

A few studies have observed no differences in the serum carnitine concentrations between VPA monotherapy and VPA with other AEDs (7, 8, 21). Unlike the previous findings (7-9), a few reported that they did not find significant differences in serum carnitine levels between patients treated with non-VPA AEDs therapy and VPA-treated patients. Both the frequency and the extent of serum carnitine were more pronounced with phenobarbital than with valproate but less pronounced with phenytoin and carbamazepine than VPA (23). Camina et al. (20) stated that non-VPA AEDs only affect the esterized form of serum carnitine, which appears to explain the absence of hypocarnitinemia in most patients treated with these drugs.

The differences in the above reported results may have been caused by some errors in methodology (9, 22), and another possibility is that patients receiving polytherapy are more severely neurologically affected, and may have an underlying metabolic disorder predisposing them to carnitine deficiency including increased renal loss of free carnitine, insufficient endogenous carnitine synthesis, or a decreased dietary carnitine intake (19-21, 24).

In the present study, there was a significant inverse correlation between the free and total carnitine levels and the duration of VPA treatment. These findings are in agreement with previous studies (7, 17, 24). Sugimoto et al. (25) considered that hepatotoxicity might occur in

cases where patients receive VPA over an extended period. Long-term administration of VPA that was conjugated to carnitine may also produce carnitine deficiency by excretion of valproylcarnitine as VPA forms an ester with carnitine (6). In contrast to these findings, Ohtani et al. (2) did not find significant correlations between them.

In this study, we found no significant correlation between the concentrations of serum carnitine and blood levels of VPA in VPA treated patients. Our results suggested that the effect of VPA on carnitine was not dose-dependent. This finding agrees with those of Laub et al. (7), Rodriguez-Segade et al. (9), Beghi et al. (17) and Thruston et al. (26), but differs from others (2, 23, 24, 27) which claimed it dose-related. Hug et al. (23) reported that, in the case of phenobarbital, there was an inverse correlation between the serum concentration of the drug and carnitine concentrations. This study, however, could not explain the inconsistent findings done in the current studies, and further investigation is needed.

We found no significant differences between serum carnitine concentrations and serum SGOT or GPT levels in VPA-treated patients. Similar observations were reported by Ohtani et al. (2), and Coulter and Allen (1). Our observations and other reports lead us to believe that carnitine deficiency was not a result of generalized hepatic dysfunction, and also could not be used as an indicator of hypocarnitinemia induced by VPA therapy.

After oral supplementation of L-carnitine in the VPA-treated patients, the concentrations of serum carnitine were significantly increased in patients showing markedly lowered serum carnitine levels. Although the clinical symptoms of carnitine deficiency were not apparent in these patients, the carnitine supplementation seemed to be effective in hypocarnitinemia induced by chronic treatment of VPA. However, Beghi et al. (17) demonstrated that impairment of carnitine metabolism and liver function by VPA does not appear to be a clinically important phenomenon, especially when administered as monotherapy to well-nourished subjects. Dreifuss et al. (27) reported that children under 2 years of age on polytherapy ran the greatest risk of developing VPA-induced fatal hepatotoxicity caused by disturbed beta-oxidation. However, Sugimoto et al. (28) considered that it might occur in older children with risk factors if VPA is administered over an extended period. Nishida et al. (29) suggested that the inhibition of beta-oxidation due to VPA administration might be relieved with L-carnitine supplementation. Murakami et al. (30) and Sugimoto et al. (28) also considered that the main purpose of supplementation of L-carnitine was to protect patients by going onto, or by already giving VPA therapy for carnitine deficiency (25, 31), and also because the onset of symptoms related to VPA-induced hepatotoxicity is sudden and unpredictable (3). In addition, this possibility is also supported by Chapoy et al. (32) and Ohtani et al. (2), who found that carnitine administration resulted in clinical improvement of recurrent Reye-like syndrome with systemic carnitine deficiency.

The question here is whether an asymptomatic decrease in the serum carnitine concentration should be treated with carnitine supplementation for the prevention of serious complications such as Reye-like syndrome (10, 33). Further studies are needed to clarify this important issue concerning the benefits of carnitine supplementation in long-term VPA treatment.

In conclusion, the results of the present investigation support that carnitine deficiency occurring in patients treated with VPA monotherapy or polytherapy is worth noting. Carnitine deficiency is related to duration of VPA treatment, not to blood levels of VPA. The activities of serum GOT/GPT in VPA treated patients can not be used as indicators of VPA induced hypocarnitinemia and/or hepatic dysfunction. Although further study is needed to clarify the question of L-carnitine supplementation, its supplementation might be of help to patients treated with VPA.

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