Original Article

Serum Carnitine Levels in Epileptic Children Before and During Treatment With Valproic Acid, Carbamazepine, and Phenobarbital

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ABSTRACT

Serum levels of free, acyl, and total carnitine were determined in 32 patients with seizures, before and after 3, 6, and 12 months of treatment with valproic acid (17 patients), carbamazepine (10 patients), or phenobarbital (5 patients). In all three treated groups, both free and total carnitine levels showed a significant decline with respect to pretreatment levels. This decline was most marked and most consistent in patients treated with valproic acid. In 35% of the patients in this group, carnitine deficiency (ie, total carnitine < 30 μmol/L) was observed by month 12. In none of the three groups were serum carnitine levels significantly correlated with the serum concentration of the drug. These findings suggest a need to monitor serum carnitine levels in children treated with any of these drugs. (*J Child Neurol* 1998;13:546–549).

Reductions in serum carnitine level have been reported frequently in patients and experimental animals treated with antiepileptic drugs.[1-14] One antiepileptic drug, valproic acid, has consistently been found to cause such a reduction.[1-7,9,11-14] However, previous results regarding the effects of other antiepileptic drugs have been less consistent.[3,6,7-10,13,14]

In most previous studies, serum carnitine levels in patients treated with antiepileptic drugs have been compared with levels in control subjects, not with levels in the same patients before treatment. This may lead to erroneous interpretations, since in some epileptic patients serum carnitine concentrations may be affected by underlying metabolic or nutritional state.[15-17]

In the work reported here, serum carnitine levels were monitored in epileptic children, before antiepileptic drug treatment and after treatment for 3, 6, and 12 months. Three antiepileptic drugs (valproic acid, carbamazepine, and phenobarbital) were used.

PATIENTS AND METHODS

Patients

Thirty-two children aged 1 to 14 years were enrolled in the study. All had been referred to the Neuropediatrics Division of the Hospital General de Galicia, between 1993 and 1996. Patients with metabolic disorders (such as aminoacidopathies, organic acidopathies, or suspected mitochondrial disease), or with serious encephalopathy or malnutrition, were excluded. The patients were divided into three groups, depending on treatment received: (1) 17 patients aged 14 months to 14 years (mean, 5.17 years), 13 with generalized seizures, including 2 with typical absence seizures, 3 with complex febrile seizures, and 1 with partial seizures, received valproic acid; (2) 10 patients aged 3 to 12 years (mean, 9.8 years), all with partial seizures, received carbamazepine; (3) 5 patients aged 1 to 2 years (mean, 1.7 years), 4 with complex febrile seizures, 1 with partial seizures, received phenobarbital.

Monitoring

Serum concentrations of total, free, and acyl (esterified) carnitine, together with blood ammonia concentration, were determined before starting antiepileptic drug treatment and at 3, 6, and 12 months after starting treatment. Blood samples were (in all cases)

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Table 1. Mean Serum Carnitine Levels Before and During Antiepileptic Therapy with Valproic Acid, Carbamazepine, or Phenobarbital

Group	Time (Months) 0	3	6	12
Valproic acid (n = 17)				
Total carnitine	44.24 (5.75)[‡]	36.12 (11.16)[§]	33.35 (9.80)[‖]	31.94 (8.37)[¶]
Free carnitine	34.35 (8.46)[‡]	29.12 (9.03)	27.00 (8.04)	25.82 (6.06)[‖]
Acyl carnitine	9.29 (5.81)	7.18 (3.54)	6.41 (4.14)	6.18 (4.50)
Mean drug level (μg/mL)		66.94	55.94	60.0
Carbamazepine (n = 10)				
Total carnitine	51.40 (8.22)	43.70 (8.86)[§]	44.80 (10.51)	42.60 (12.14)
Free carnitine	46.50 (8.51)	39.30 (8.62)	37.60 (7.96)[§]	34.30 (8.27)[‖]
Acyl carnitine	4.90 (2.92)	4.40 (2.91)	7.10 (4.25)	8.30 (5.79)
Mean drug level (μg/mL)		7.05	6.29	7.13
Phenobarbital (n = 5)				
Total carnitine	48.40 (3.21)[†]	34.40 (8.17)[§]	37.40 (3.21)[§]	33.80 (4.71)[‖]
Free carnitine	40.40 (5.94)[*]	31.40 (11.57)	32.80 (2.17)	27.80 (4.44)[‖]
Acyl carnitine	8.00 (7.38)	3.00 (3.46)	4.60 (3.51)	6.40 (3.78)
Mean drug level (μg/mL)		18.56	19.67	18.75
Controls (n = 71)				
Total carnitine	55.37 (9.40)			
Free carnitine	49.00 (5.92)			
Acyl carnitine	6.30 (2.50)			

Statistically significant differences with respect to the corresponding control-group mean: [*] = $P < .05$; [†] = $P < .01$; [‡] = $P < .001$. Statistically significant differences with respect to the corresponding pretreatment mean: [§] = $P < .05$; [‖] = $P < .01$; [¶] = $< .001$.
Standard deviations are shown in parentheses.

obtained with informed parental consent, early in the morning after fasting for 8 hours.

As healthy-subject controls, we used data for total, free, and acyl carnitine levels in 71 healthy children from our region (aged 1 to 16 years, mean 9.5 years).[9] In view of carnitine levels in this control group, "carnitine deficiency" was taken to be indicated by a total carnitine level of less than 30 μmol/L, and "carnitine insufficiency" by an acyl-to-free-carnitine ratio of more than 0.25.[18]

In the serum samples taken after starting treatment, antiepileptic drug levels were also determined. Minimum concentrations were 30 μg/mL valproic acid, 5 μg/mL carbamazepine, and 15 μg/mL phenobarbital; mean concentrations are shown in Table 1. In all patients, seizures were fully controlled by the corresponding drug treatment. None of the patients exhibited side effects necessitating withdrawal of therapy.

Analytical Procedures

Morning blood was collected into dry tubes, and one part was centrifuged for 10 minutes at 4°C within 5 minutes, to separate serum. Serum was stored at –20°C unless assayed immediately. Free carnitine was determined by the method of Rodríguez-Segade et al.[19] Total carnitine (ie, the sum of free and acyl carnitine) was determined by the same procedure after acyl carnitine had been hydrolyzed with 1 mol/L of KOH, as per McGarry and Foster.[20] Acyl carnitine concentration was estimated as the difference between free carnitine concentration (ie, before alkaline hydrolysis) and total carnitine concentration.

Ammonia concentration was measured by the microdiffusion method. The sample is mixed with a dried alkaline buffer (Na_2CO_3-$NaHCO_3$, pH 10.3); the ammonia released diffuses through a gas-permeable polypropylene film and reacts with a pH indicator (Bromocresol Green). The color of the product was measured with a reflectance meter (Blood Ammonia Checker System, Kyoto Daiichikagaku Co.).

Serum valproic acid, carbamazepine, and phenobarbital levels were determined in a TDX analyzer with reagents from Abbott Laboratories (North Chicago, IL).

Statistical Analysis

The statistical significance of within-subject variations in carnitine levels over time was investigated with Friedman's tests. To compare treated-group pretreatment mean carnitine levels with control-group mean carnitine levels, the Kruskall-Wallis test was used. Possible relationships between basal carnitine level and type of seizure were investigated with the Wilcoxon test. Possible correlations between mean pretreatment carnitine levels and age, and between during-treatment carnitine levels and drug levels in serum, were evaluated by Spearman rank correlation analysis. Unless otherwise stated, statistical significance is taken to be indicated by P values of less than .05.

RESULTS

The time-courses of mean carnitine levels in the different groups are summarized in Table 1.

Neither mean free carnitine, mean acyl carnitine, nor mean total carnitine levels measured before treatment in the carbamazepine-treated group differed significantly from levels in the control group. However, both mean free carnitine and mean total carnitine levels were significantly

lower in the valproic acid- and phenobarbital-treated groups than in the control group. Nevertheless, in no case were carnitine levels low enough for classification as insufficient. The significant differences between the valproic acid-treated group and the control group, and between the phenobarbital-treated group and the control group, were not related to type of seizure. Furthermore, pretreatment carnitine levels showed no significant correlation with age, in either the valproic acid- or the phenobarbital-treated group.

In the valproic acid-treated group, serum levels of both free and total carnitine declined steadily over the treatment period. Mean total carnitine level was significantly lower than the mean pretreatment level at all times of measurement, whereas mean free carnitine level was only significantly lower after 12 months (Table 1). After 12 months, the drop in free/total carnitine levels (with respect to the pretreatment level) was more than 50% in three patients (18%), and the acyl-to-free carnitine ratio was greater than 0.25 in four patients (24%). Carnitine deficiency was reached within 3 months in two patients (12%), within 6 months in three patients (18%), and within 12 months in six patients (35%).

In the carbamazepine-treated group, free and total carnitine levels were both lower after 3 months of treatment than before treatment, though the difference was statistically significant only in the case of total carnitine. Subsequently, total carnitine levels remained more or less unchanged, while free carnitine dropped steadily and showed mean values that were significantly lower than before treatment. Simultaneously, mean acyl carnitine level gradually increased, although at no point were mean acyl carnitine levels significantly higher than before treatment. After 12 months, the acyl-to-free carnitine ratio was greater than 0.25 in three patients (30%). Carnitine deficiency was not observed in any of the patients.

In the phenobarbital-treated group, total carnitine level declined gradually over the treatment period, the difference being statistically significant from 3 months after starting treatment onwards. The initial decline was due to a decline in both free and acyl carnitine levels. Subsequently, however, the acyl carnitine level started to increase again, whereas the free carnitine level declined steadily, and indeed by month 12 was significantly lower than before treatment. After 12 months, the acyl-to-free carnitine ratio was greater than 0.25 in one patient (20%). Carnitine deficiency was not observed in any of the patients.

Ammonia levels remained normal in the patients treated with carbamazepine or phenobarbital. In the valproic acid-treated group, however, three patients showed marked increases within 3 months of treatment (from 50 to 123 ng/dL, from 34 to 95 ng/dL, and from 40 to 87 ng/dL), while one patient showed a marked increase by month 12 (from 50 to 89 ng/dL). In this patient, the increase in serum ammonia level coincided with a decline in total carnitine to a level defined as deficient (23 μmol/L).

Neither free, acyl, or total carnitine levels, nor ammonia levels, were significantly correlated with serum drug level in any of the three groups.

DISCUSSION

The present results indicate that a drop in serum concentrations of free and total carnitine (with respect to pretreatment levels in the same patient) occurs not only in patients treated with valproic acid, but in patients treated with carbamazepine or phenobarbital. The extent of the drop was in no case correlated with drug level in serum. This is in contrast with the results of Ohtani et al,[1] Opala et al,[5] Thom et al,[6] and indeed with the results of a previous study by our group.[9] In none of these studies were either carbamazepine or phenobarbital found to cause reduced serum carnitine levels. Zelnik et al,[21] in a study of valproic acid, carbamazepine, and phenobarbital, found that only valproic acid caused reductions in free and total carnitine (with respect to pretreatment levels). Results similar to those of the present study have, however, been obtained in other studies of patients undergoing long-term treatment, namely the studies by Rodríguez-Segade et al[8] in epileptic adults, and by Hug et al[7] and ourselves[10] in epileptic children.

Although we found that all three drugs led to reduced carnitine levels, there were a number of differences in the form of the response. Over the 12-month study period, valproic acid had a constant and gradual effect on free and total carnitine levels, without having any effect on acyl carnitine level. This latter finding contrasts with those of Riva et al,[22] who found that valproic acid treatment increased acyl carnitine levels in a longitudinal study of both adults and children. We found that carbamazepine led to an initial decline in total carnitine level, which subsequently remained more or less constant. However, carbamazepine had a more gradual effect on free carnitine, which was still dropping by month 12, and on acyl carnitine, which rose throughout the treatment period. Phenobarbital induced an initial decline in total carnitine concentration, in parallel with a decline in both free and acyl carnitine; by the end of the treatment period, total carnitine remained low, but by this stage the low level was entirely attributable to the low level of free carnitine.

All three drugs caused carnitine insufficiency (high acyl-to-free carnitine ratio; see Methods) in some patients, but carnitine deficiency (low total carnitine; see Methods) was observed only in valproic acid-treated patients. A drop of more than 50% in total carnitine level was observed only in valproic acid-treated patients. Furthermore, increased blood ammonia levels were observed only in valproic acid-treated patients. That valproic acid has such effects in a subset of patients has been noted previously,[1,5,8,9,21,22] and possibly indicates that some individuals are particularly sensitive to the effects of valproic acid on carnitine stores. The clinical consequences of these effects are not clear, since all patients showing some or all of these effects responded well to the therapy with regard to seizure control and lack of symptomatic side effects.

Statistically significant differences in pretreatment levels of free and total carnitine were observed both between the valproic acid-treated patients and the control subjects,

and between the phenobarbital-treated patients and the control subjects. These differences did not show any significant relationship to type of seizures. Neither can the relatively low carnitine levels in the valproic acid- and phenobarbital-treated patients be attributed to nutritional problems, since patients with malnutrition were excluded from the study. One possible explanation is that the low carnitine levels in these groups are related to the relatively young age of these patients (mean, 5.2 years in the valproic acid group and 1.7 years in the phenobarbital group, versus 9.8 years in the carbamazepine group and 9.5 years in the control group); we did not find any significant correlation between age and serum carnitine levels, but such a correlation (levels increasing with age) has been detected in a previous large-sample study.[23]

The present results thus suggest that there is a need to monitor serum levels of free and total carnitine not only in children undergoing treatment with valproic acid, but also in children undergoing treatment with carbamazepine or phenobarbital, as pointed out previously by Rodríguez-Segade et al[8] with regard to adults, and by ourselves[10] with regard to children. Monitoring ideally should begin before treatment starts, so that subjects may act as their own controls; this is important, since serum carnitine levels may differ from one individual to another because of factors including age and nutritional and metabolic status.[15–17,23,24] If carnitine deficiency or insufficiency is observed, one possibility is to administer an oral L-carnitine supplement. This approach is widely considered to be effective for treating valproic acid-induced hypocarnitinemia,[1,9,25] although some authors have disagreed.[17] In such patients, carnitine supplementation counteracts the inhibition by valproic acid of uptake of carnitine by cells, buffers excess (and potentially toxic) acyl-CoA, and increases intramitochondrial free CoA, thereby mitigating mitochondrial dysfunction.[26] Further large-sample studies of the effects of antiepileptic drugs on carnitine levels in children with different types of seizure may help to elucidate these questions.

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