Valproate Therapy Does Not Deplete Carnitine Levels in Otherwise Healthy Children

Shinichi Hirose, MD; Akihisa Mitsudome, MD; Sawa Yasumoto, MD; Atsushi Ogawa, MD; Yukiko Muta, BS; and Yasuko Tomoda, MD

ABSTRACT. Objective. To determine whether children with epilepsy undergoing valproate (VPA) antiepileptic therapy and who are otherwise healthy have a lower serum level of carnitine (CAR) and a higher plasma level of plasma ammonia than do normal children.

Methodology. A total of 45 children with epilepsy, 6.3 to 21.7 years of age, who were treated solely with VPA and were free of abnormal neurologic findings or nutritional problems were randomly selected (VPA-treated group). An age-matched control group (n = 45) was selected from subjects without epilepsy (control group). Total (T) and free (F) serum CAR, serum VPA concentration, and the plasma ammonia level were measured and analyzed.

Results. Serum VPA concentration exhibited a weak negative correlation with both T- (r = -0.34) and F-CAR (r = -0.41). The T-CAR levels were 55.7 ± 12.4 and 57.6 ± 12.1 mM, and the F-CAR levels 42.7 ± 9.9 and 44.4 ± 9.9 mM in the VPA-treated and control groups, respectively. Thus, there was no significant difference in T- or F-CAR levels between the VPA-treated and control groups. Plasma ammonia levels were the same in the two groups: 26 ± 9.2 and 29.4 ± 11.8 mM in the VPA-treated and control groups, respectively. There was no significant correlation between blood ammonia and either T- (r = +0.024) or F-CAR (r = -0.026).

Conclusion. Children on a regular diet ingest a sufficient amount of CAR that more than meets their daily CAR requirement. The level of neither T- nor F-CAR in patients with epilepsy and without severe neurologic or nutritional problems being treated with VPA appeared to be affected by VPA therapy. Because the blood CAR level depends on nutritional condition rather than on blood VPA concentration, CAR deficiency caused by VPA is not likely to occur in this population. The usefulness of supplementation of CAR for this type of patient with epilepsy, therefore, must be reevaluated carefully. Pediatrics 1998;101(5). URL: http://www.pediatrics.org/cgi/content/full/101/5/e9; carnitine, valproate, valproic acid, epilepsy, liver dysfunction, hyperammonemia, lipid metabolism, Reye's syndrome, handicap, malnutrition.

Abbreviations. VPA, valproate; CAR, carnitine; F-CAR, free carnitine; A-CAR, acyl carnitine; T-CAR, total carnitine.

Valproate (VPA) is an antiepileptic drug bearing a carboxyhydroxy base and is thus structurally a short-chain fatty acid or an organic acid. VPA has a wide spectrum of antiepileptic effects. Therefore, the indications for VPA therapy are now expanding to include many different types of epilepsy beyond absence (epilepsy), for which VPA was first approved by the US Food and Drug Administration. A major fraction of ingested VPA undergoes glucuronol conjugation in the liver. Some of the fractions of VPA are excreted in the urine. As an organic acid, VPA can be excreted in the urine as a complex with carnitine (CAR), ie, valproyl-CAR, although unconjugated VPA also can be excreted in the urine. CAR is a nutrient supplied primarily by meat and dairy products. CAR also is synthesized de novo in the liver and stored primarily in muscle. A small fraction of CAR in the body, ~0.6%, is found in the blood. Most of blood CAR exists as free (F)- CAR, whereas the rest exists as acyl (A)- CAR. The sum of the two fractions is referred to as total (T)- CAR. The main function of CAR is to transport fatty acids from the cytosol to the inner compartment of the mitochondria where fatty acids are used for energy production by β-oxidation. CAR also facilitates the urinary excretion of organic acids that precipitate in the mitochondria. Thus, CAR has an important role in energy production in the body as well as in the maintenance of mitochondrial metabolism.

When an excess amount of organic acid accumulates in the body, such as in organic aciduria, CAR can be depleted because it is coexcreted with organic acids in the urine. Systemic CAR deficiency manifests itself as hyperammonemia, hypoglycemia, and other severe metabolic conditions seen in Reye-like syndrome.

In the past decade, several anecdotal reports have been published concerning metabolic insufficiencies associated with VPA therapy, ie, hyperammonemia and Reye-like syndrome. The depletion of CAR during VPA treatment also has been demonstrated. The mechanism of metabolism of VPA suggests the possibility that VPA might reduce CAR level. Therefore, supplementation of an active form of CAR, L-CAR, during VPA therapy has been recommended by some groups. It is uncommon, however, for patients with epilepsy seen in daily clinical practice to suffer from manifestations of CAR depletion attributable to VPA, unless they have severe neurologic complications or nutritional problems.

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In this study, serum CAR levels were measured in children with epilepsy who have no severe neurologic complications or nutritional problems to determine whether VPA indeed reduces their blood CAR levels, as reported previously.

**MATERIALS AND METHODS**

**Subjects**

This study was performed at the Pediatric Neurology Clinic of Fukuoka University Hospital from January 1994 to December 1995. The patients who were to undergo CAR measurement (VPA-treated group) were selected consecutively during the study period if they met the following criteria: 1) The diagnosis of epilepsy was made based on both electroencephalographic findings and clinical manifestations; 2) VPA therapy was the sole treatment for epilepsy; 3) VPA therapy had continued for >6 months before CAR measurement; 4) subjects had normal intelligence and neurologic examination findings; 5) both weight and height of subjects were within ± 2 SD units of the mean for age; 6) diet was regular and given orally.

A total of 45 patients (20 males, 25 females) met the criteria during the study period. Age ranged from 6.3 to 21.7 years, with a mean of 13.1 ± 3.9 years. Patients’ compliance with medication was evaluated by measurement of blood VPA concentration at least once before CAR measurement. Because previous VPA concentrations of the subjects were within appropriate limits for epilepsy treatment, all subjects entered in this study were judged to have good compliance with medication.

An age-matched control group was chosen from outpatients who visited the General Pediatric Clinic at Fukuoka University Hospital with a minor illness such as upper respiratory tract infection or other similar condition during the same period and who met criteria 4 to 6 described above. The control group consisted of 19 males and 26 females. The age of this group ranged from 6.0 to 17.1 years, with a mean of 11.8 ± 2.6 years. There was no significant difference between the two groups in age or proportions by gender. Neither the VPA-treated nor the control group included any patient with organic acidemia or other metabolic disorder. No patient in either group had been treated with antibiotics containing pivalic acid, which is known to decrease blood CAR level.20,21

**CAR Measurement and Other Blood Tests**

T- and F-CAR were determined with a Cobas Fara automatic analyzer using an enzyme-cycling method with and without the addition of A-CAR esterase, respectively.20 A-CAR was calculated using an enzyme-cycling method with and without the addition of A-CAR esterase, respectively.20 A-CAR was calculated by subtracting T-CAR from F-CAR. The reagents used for measurement were obtained as a kit from Kainos Laboratories, Inc, and manufactured by Asahi Kasei Chemical Industrial Co, (Tokyo, Japan). Blood was obtained by venipuncture without a tourniquet 2 hours after the administration of VPA, when breakfast was eaten. For the control group, blood was obtained in the same manner as for the VPA-treated group, and the time of the blood sampling was 2 hours after breakfast. The serum was stored at −20 °C until the time of measurement.

The blood ammonia level was determined for heparinized plasma with a Hitachi automatic analyzer by an enzyme assay using glutamate dehydrogenase. The plasma was prepared immediately after blood sampling and handled at 4 °C throughout.

Statistical analyses were performed using student’s t test.

**RESULTS**

Table 1 summarizes the subjects of the VPA-treated and control groups. As planned, there was no significant difference between the two groups in number of subjects, age, or gender distribution. Blood VPA concentration in the VPA-treated group ranged from 27 to 112 mg/mL, with a mean of 53.7 ± 18.2 mg/mL. These concentrations were comparable to the value recommended for epilepsy treatment and indicated both compliance with medication as well as appropriate performance of VPA therapy.

To examine whether serum CAR level decreased as blood VPA concentration increased, the correlation between blood VPA and CAR levels was determined for the VPA-treated group. A weak negative correlation was found between VPA concentration and both T- (r = −0.34; P < .05) and F- (r = −0.40; P < .01) CAR levels (Fig 1). However, even with the higher valproate concentrations, both T- and F-CAR levels were maintained within the reference range for CAR.

Figure 2 shows serum T- and F-CAR levels in the VPA-treated and control groups. T-CAR levels were 55.7 ± 12.4 mM in the VPA-treated group and 57.7 ± 12.1 mM in the control group; this difference was not significant (P > .05). F-CAR levels were 42.7 ± 9.9 and 44.4 ± 9.9 mM in the VPA-treated group and control groups, respectively; this difference also was not significant (P > .05). The ratio of F- to T-CAR, which is known to significantly affect maintenance of mitochondrial function, was calculated. The ratios were 0.77 ± 0.10 and 0.78 ± 0.07 in the VPA-treated and control groups, respectively. As expected from the CAR levels, this difference was not significant (P > .05).

The blood ammonia level is believed to be an indicator of the mitochondria dysfunction caused by CAR deficiency. Hyperammonemia is thought to be a typical manifestation of secondary CAR deficiency such as that in organic acidemia. There also are anecdotal reports of the hyperammonemia during VPA therapy. To examine whether the VPA-treated group had higher blood ammonia levels, we compared blood ammonia levels in the two (Fig 3); they were 26.0 ± 9.2 mM for the VPA-treated group and 29.4 ± 11.8 mM for the control group. This difference was not significant (P > .05).

To directly examine the correlation between serum CAR and blood ammonia levels, we tested whether subjects with lower levels of CAR have higher blood ammonia levels. All CAR and blood ammonia levels obtained from both the VPA-treated and control groups are plotted together in Fig 4. No correlation was observed between blood ammonia level and either T- (r = −0.024) or F-CAR (r = +0.026) level.

**DISCUSSION**

Although CAR can be synthesized de novo in the liver, the intake of exogenous CAR still is a main source of this nutrient for humans.21 For healthy adults, an average of 100 to 300 mg of CAR is ingested per day, primarily from meat and dairy products. When added to endogenous CAR, this amount is far beyond the daily requirement of CAR, because the capacity for storage of CAR in the body is ∼100 mmol. Some 95% of CAR in the human body is stored in the muscle.4,5 In assessing blood CAR level,
the nutritional condition and amount of muscle of subjects therefore must be taken into account. Previous reports of CAR deficiency during VPA therapy did not necessarily consider the nutritional status of subjects studied. The first report of CAR depletion during VPA therapy was indeed made for patients with severe neurologic complications. Although the nutritional status of these patients was not well described, it is postulated that they were at nutritional risk.

In contrast, the population of patients with epilepsy we studied were well nourished and within the normal body weight and height range for the corresponding age groups. Furthermore, the subjects examined in the present study were with epilepsy but had no other neurologic findings and were not mentally retarded. These complications, which frequently accompany epilepsy, might have compromised their ability related to nutritional status, oral feeding disability, or enteral feeding difficulties resulting from recurrent esophagitis caused by gastroesophageal reflux.

For this population, weak correlations between VPA concentration and T- and F-CAR levels were found. This finding supports the hypothesis that VPA reduces blood CAR and is consistent with previous reports. In contrast to most previous studies of blood CAR levels during VPA therapy, however, both blood T- and F-CAR levels in the VPA-treated group were comparable with levels in the control group. Furthermore, the plasma ammonia levels did not increase in the VPA-treated group.

In the past decade, VPA has become one of the most important drugs in epilepsy. Potential hazards of CAR deficiency caused by VPA therefore were communicated to many physicians, with considerable impact. Nevertheless, even for neurologists who treat either adults or children and who prescribe VPA regularly to their patients with epilepsy, it is uncommon to encounter the manifestations resulting from CAR deficiency solely attributable to VPA therapy.

VPA is a short-chain fatty acid, and some fractions of ingested VPA can be excreted in the urine as valproyl CAR. Therefore it is theoretically possible and appears rational that VPA administration causes CAR deficiency, as occurs in organic acidemia by the same mechanism. In animal experiments performed primarily with rats, CAR was depleted in a dose-dependent manner by VPA administration, although the doses of VPA used were considerably higher than were clinical doses.

http://www.pediatrics.org/cgi/content/full/101/5/e9

Fig 1. Correlation between serum carnitine and valproate levels. T- and F-CAR levels in the VPA-treated group were measured by the enzyme-cycling method. The blood valproate concentrations also were determined using aliquots of the same serum. The correlation between CAR and VPA levels was determined. Open circles indicate T-CAR, and solid triangles indicate F-CAR. The correlations between VPA and T- and F-CAR are indicated by a dashed line ($r = -0.34; P = 0.025$) and a solid line ($r = -0.41; P = 0.003$), respectively.

Fig 2. Comparison of serum CAR levels in VPA-treated and control groups. T- and F-CAR levels were measured in the VPA-treated and control groups. T-CAR levels (open bars) were 55.7 ± 12.4 and 57.6 ± 12.1 mM ($P > 0.05$), and F-CAR levels (shaded bars) were 42.73 ± 9.9 and 44.42 ± 9.9 mM ($P > 0.05$) in the VPA-treated and control groups, respectively.

Fig 3. Comparison of plasma ammonia levels between VPA-treated and control groups. Plasma ammonia levels were determined in the VPA-treated and control groups, and were 26.0 ± 9.2 mM (shaded bar) and 29.4 ± 11.8 mM (open bar), respectively. This difference was not significant ($P > 0.05$).
In the metabolism of physiologic doses of VPA, the majority of ingested VPA is conjugated with glucuronide and then undergoes various processes of degradation in the liver as VPA-glucuronide. A small portion of the ingested VPA is processed by β-oxidation. Furthermore, VPA itself can be excreted in the urine without CAR. A recent analysis of urinary VPA during VPA therapy has disclosed that valproyl CAR comprises only 0.1% of the total VPA and 1% of the total amount of A-CAR in the urine.23 This finding indicates that urinary excretion of CAR along with VPA cannot be sufficient to deplete CAR as long as VPA is used at pharmacologic doses. This finding for urinary VPA and CAR levels is in contrast to levels in patients with organic acidemia and those receiving antibiotics containing pivalic acid. In organic acidemia, a considerable amount of A-CAR formed with the organic acids that accumulate in the body is found in the urine.8,9 A-CAR excretion in the urine not only consumes CAR but also interferes with the reabsorption of CAR in the microtubules of the kidney.24 These mechanisms synergistically induce CAR deficiency in patients with organic acidemia. The same pathology has been observed in the interaction between blood CAR depletion and pivalic acid contained in antibiotics used from both groups were plotted together. Open circles indicate total CAR, and solid triangles indicate F-CAR. The correlations between ammonia level and T-CAR (dashed line, $r = +0.040$) and F-CAR (solid line, $r = -0.026$) were not significant.

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REFERENCES


Fig 4. Correlation between serum CAR and plasma ammonia levels. The serum CAR and plasma ammonia levels in the VPA-treated and control groups were measured. All CAR and blood ammonia levels obtained from both groups were plotted together. Open circles indicate total CAR, and solid triangles indicate F-CAR. The correlations between ammonia level and T-CAR (dashed line, $r = +0.040$) and F-CAR (solid line, $r = -0.026$) were not significant.
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