L-Carnitine supplementation to reverse hyperammonemia in a patient undergoing chronic valproic acid treatment: A case report

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Abstract
Valproic acid is a broad-spectrum anticonvulsant that has also gained attention in the psychiatric setting. With respect to safety, valproic acid may induce a seemingly rare condition, hyperammonemia, which can induce a wide variety of symptoms ranging from irritability to coma. The proposed mechanism of hyperammonemia involves depletion of carnitine and overproduction of a toxic metabolite, 4-en-valproic acid, both of which impair the urea cycle and thus ammonia elimination. Carnitine is a commonly used antidote for acute intoxication of valproic acid, but is not a therapeutic option for management of chronic adults with adverse effects related to valproic acid. We herein report a case involving a woman with epilepsy who developed hyperammonemia after a change in her anticonvulsant therapy. She reported increased seizures and gastrointestinal disturbances. Her ammonia, valproic acid, 4-en-valproic acid, and carnitine levels were monitored. Her ammonia level was elevated and her carnitine level was at the inferior limit of the population range. She was supplemented with carnitine at 1 g/day. After 1 month, her ammonia level decreased, her carnitine level increased, and her seizures were better controlled. Carnitine supplementation was useful for reversal of her hyperammonemia, allowing her to continue valproic acid for seizure control.

Keywords
Valproic acid, hyperammonemia, carnitine

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Introduction
Valproic acid (VPA) is a widely used broad-spectrum anticonvulsant.1 In the last several years, it has also gained attention in the
psychiatric setting as a mood stabilizer. It is usually well tolerated, although hepatic function impairment necessitates tight control in children. VPA has also been associated with metabolic disorders leading to weight gain and occasionally with hyperammonemia. The latter may develop asymptptomatically or lead to encephalopathy. The prevalence of symptomatic hyperammonemia caused by VPA is unknown, and the condition is thought to be rare in adults. However, in a study carried out by our group, almost one-third of adult patients (8 of 28 patients) developed ammonia levels higher than the reference range, although only one of these patients exhibited unequivocal symptoms (encephalopathy). L-Carnitine (LCAR) is commonly used as an antidote in acute intoxication with VPA, but its use in chronic treatment is reserved for the pediatric setting.

We herein report a case in which VPA-induced hyperammonemia during chronic treatment was reverted by the use of LCAR as adjuvant therapy.

Case report

A 42-year-old woman had an 18-year history of seizures. She was a current smoker. In 1995, she first developed focal somatosensory seizures (paresthesia in the right limbs); these evolved to dysphasic seizures and loss of consciousness. Neuroimaging revealed a left temporoparietal arteriovascular malformation. Her primary pharmacological treatment was carbamazepine (600 mg/day) and VPA (1500 mg/day); however, the seizures persisted. The carbamazepine was stopped and lamotrigine (LTG) was added at 300 mg/day. After several months of treatment, the patient presented with increased seizures, cephalgia, and nausea and was referred to our service for monitoring. Her hepatic and renal function was normal. At that time point, she was receiving 1500 mg of VPA. Her morning trough VPA level was measured using a previously described validated high-performance liquid chromatography (HPLC) technique with minor modifications, and her 2-propyl-4-pentenoic acid (4-en-VPA) level was determined using the same technique. Thirty microliters of internal standard (octanoic acid) was added to 1 mL of plasma. A Phenomenex Luna CN 5 μm column (150 × 4.6 mm) was used for the stationary phase. The mobile phase was a mixture of potassium phosphate monobasic (40 mM, pH 3.4) and acetonitrile (90/10) pumped at a flow rate of 1.5 mL/min. The column compartment was kept at 36°C, and the wavelength detection was 210 nm. Under these conditions, the retention times of the analytes were 3.8, 4.9, and 6.8 min for 4-en-VPA, VPA, and octanoic acid, respectively. The HPLC method was linear between 1.1 and 133 mg/L for VPA and between 0.78 and 16.5 mg/L for 4-en-VPA. The within-day and between-day precision (coefficient of variation) for the low, intermediate, and high concentration of both analytes was <15%. The accuracy at the same concentrations was within 92% and 108%. The blood ammonia concentration was determined with a Cobas c311 (Roche Laboratories), and LCAR and its acyl derivatives were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Extraction was performed on 3.2-mm filter paper disks punched out from dried blood spot specimens using 100% methanol solution containing the internal standards. The internal standards used were the Cambridge isotope internal standards NSK sets A and B, which contained the following stable isotopes: acylcarnitines d9-C0, d3-C2, d3-C3, d3-C4, d9-C5, d3-C8, d9-C14, and d3-C16. The extracted samples underwent derivatization with 3 N butanolic HCl at 65°C and were finally reconstituted in an acetonitrile/water (50/50) solution containing 0.02% formic acid (mobile phase). The samples were
analyzed with HPLC-MS/MS (Dionex-AB Sciex 3200 triple quadrupole) using the precursor ion spectra of 85 m/z. The results are depicted in Table 1.

The LCAR levels were minimal according to the population reference ranges and as evidenced by a high acylcarnitine/LCAR (ACYLCAR/LCAR) ratio of >0.4, which is a good biomarker of LCAR deficiency. Because of the patient’s high ammonia concentration (>94 mg/mL), LCAR was added at 1 g/day. The patient was then monitored 2 months later at the doctor’s request; the results are shown in Table 1. With addition of the LCAR, the ammonia level decreased to within the reference range. The metabolite concentration also decreased, while the LCAR level increased after supplementation, as expected, from a borderline-low concentration to a normal value. Finally, the ACYLCAR/LCAR ratio decreased due to resolution of the LCAR deficiency.

This study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki and was approved by the Ethics Review Committee of the Faculty of Chemistry (Uruguay). The patient provided written informed consent for publication.

Table 1. Effect of LCAR supplementation at 1 g/day

|                      | Without LCAR | With LCAR |
|----------------------|--------------|-----------|
| Blood VPA (mg/L)     | 86.6         | 46.3      |
| 4-en VPA (mg/L)      | 3.6          | 0.7       |
| 4-en-VPA/VPA         | 0.042        | 0.015     |
| metabolic ratio      |              |           |
| Ammonium (NH₄⁺) (µg/dL) | 295         | 75        |
| LCAR (µmol/L)        | 20.9         | 29.3      |
| ACYLCAR/LCAR ratio   | 0.45         | 0.23      |

LCAR, L-carnitine; VPA, valproic acid; ACYLCAR/LCAR, acylcarnitine/L-carnitine

Discussion

VPA has been on the market for almost 40 years now. It was initially considered to be a safer alternative to first-generation anticonvulsants and free of serious side effects. However, some reports have associated VPA with fulminant hepatitis, a Reye-like syndrome, and encephalopathy. Although hyperammonemia has also been reported, little attention has been paid to its prevalence. One report stated that up to 50% of patients taking VPA developed hyperammonemia, although they were asymptomatic. With respect to its pharmacokinetics, VPA is highly protein-bound and undergoes extensive liver metabolism. During its elimination, VPA follows three main routes: glucuronidation (50%), β-oxidation in the mitochondria (40%), and ω-oxidation (10%); the latter leads to formation of a toxic metabolite, 4-en-VPA. This metabolite impairs the function of carbamoyl phosphate synthetase (CPS), which catalyzes the conversion of ammonia in the first step of the urea cycle. Consequently, the ammonia level rises. However, this is not the only factor affecting ammonia elimination. In the β-oxidation pathway, VPA must cross the mitochondrial membrane via the carnitine shuttle, as fatty acids do. LCAR depletion may occur in patients receiving high doses of VPA or who experience acute intoxication, which may in turn result in less β-oxidation of fatty acids (including VPA), less acetyl-CoA production, and decreased synthesis of N-acetyl glutamic acid, an allosteric activator of CPS. As β-oxidation decreases, ω-oxidation of VPA is favored and higher levels of 4-en-VPA are formed, which enhances CPS inhibition as described above.

A well-established risk factor for hyperammonemia in the neurological literature is the combination of VPA with other antiepileptic medications, particularly...
phenobarbital, phenytoin, and carbamazepine. The mechanisms of action are thought to be related to an increase in the production of toxic VPA metabolites because all of them are inducers of the ω-oxidative pathway.\textsuperscript{16} Case reports have also raised the possibility that hyperammonemia results from an interaction between the effects of VPA and topiramate\textsuperscript{17} and VPA and LTG.\textsuperscript{18}

In our patient, no VPA or ammonia monitoring was performed while she was taking carbamazepine and VPA. LTG is mainly eliminated by glucuronidation and competes with VPA for this metabolic route. The addition of LTG may have enhanced deviation of VPA metabolism to oxidative routes favoring the production of 4-en-VPA (by ω-oxidation) because β-oxidation was impaired by LCAR depletion.\textsuperscript{19} All of these processes in combination could have led to the development of hyperammonemia, the consequences of which (seizures) are difficult to differentiate from the pathology itself (epilepsy) and that can be misdiagnosed as therapeutic failure instead of an adverse drug reaction related to the use of VPA. Hence, seizures might be seen as the final step of the excitatory symptoms of hyperammonemia due to the increase in glutamate neurotransmitter synthesis.\textsuperscript{20}

Exogenous supplementation of LCAR has been proposed to prevent the development of hyperammonemia. In Uruguay, however, this strategy has only been applied in the pediatric setting. Collectively, the available evidence provides a reasonable argument for the role of LCAR supplementation in certain cases of childhood-onset epilepsy.\textsuperscript{21,22} However, well-designed studies of LCAR replacement therapy in children with epilepsy are still needed. LCAR supplementation was useful for resolution of our patient’s VPA-related adverse drug reaction. This is important knowledge for clinicians, who might otherwise change the therapeutic strategy in such patients.

LCAR supplementation not only favors ammonia elimination but also restores VPA metabolism to normal routes, increasing β-oxidation and thus decreasing ω-oxidation and therefore 4-en-VPA formation. The metabolic ratio decreased after LCAR addition. This can be explained by the higher impact that the decrease in carnitine has on the kinetics of 4-en-VPA, which is also a fatty acid. The ACYLCAR/LCAR ratio also decreased and was maintained within the reference ranges reported in the literature.\textsuperscript{11} The ammonia level also returned to the reference range (25–94 μg/mL).

Wide variation exists in the doses and routes of administration of LCAR, making it challenging to identify the optimal dosing strategy.\textsuperscript{23} A dose of 1 g/day was proposed as the initial dose in the present case, but as the patient’s condition improved, this dose was maintained as long as the VPA treatment continued.

The patient’s renal and liver function remained normal despite her elevated ammonia, VPA, and 4-en-VPA levels. Monitoring of the ammonia level is advisable in adult patients undergoing chronic VPA treatment, and LCAR supplementation could be a solution to this VPA-associated adverse drug reaction.

The main limitation of this study is that the case report included only one patient. Despite the usefulness of this intervention, more patients still need to be studied to draw stronger conclusions.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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