A 9-Month-Old Boy with Seizures and Discrepant Urine Tryptophan Concentrations

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CASE

A 9-month-old boy with a history of seizures underwent a neurologic and biochemical-genetic evaluation. The brain MRI results were compatible with a diagnosis of Leigh disease, also known as subacute necrotizing encephalomyelopathy, a rare neurometabolic disorder that affects the central nervous system. The patient had been prescribed several antiepileptic medications, including levetiracetam, lamotrigine, phenobarbital, vigabatrin, and topiramate. Metabolic screening for free amino acids was performed on the child’s urine, with concentrations quantified with an automated amino acid analyzer (Hitachi L-8800). This commercially available system couples ion-exchange liquid chromatography with postcolumn ninhydrin derivatization before UV detection. This analysis revealed a very large peak with a retention time consistent with the elution of tryptophan (Fig. 1). The calculated urinary excretion was 125 204 μmol/g creatinine. In addition, the urinary concentrations of γ-aminobutyric acid (GABA),5 β-alanine, β-aminoisobutyric acid, and glutamine were also increased substantially. The concentrations of the remaining amino acids were within their respective reference intervals. For confirmation, we submitted a urine aliquot to an external reference laboratory for analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS). This analysis revealed a tryptophan excretion of 71 μmol/g creatinine (reference interval, 15–30 μmol/g creatinine).

DISCUSSION

The gross discrepancy in tryptophan concentration between the 2 assays, combined with the increased urinary excretion of GABA, β-alanine, β-aminoisobutyric acid, and glutamine, triggered an inquiry into the potential causes. The patient had a neonatal-onset seizure disorder, hypotonia, a developmental delay, and MRI findings compatible with Leigh disease, but isolated hypertryptophanuria is not a finding for this disorder. In addition, the overall prognosis of patients with Leigh disease is poor. The disease represents the clinical and radiologic expression of a group of inherited disorders of energy metabolism, specifically disorders of oxidative phosphorylation or pyruvate oxidation. The ultimate cause of Leigh disease is a failure of ATP production or energy metabolism. Leigh syndrome is diagnosed on the basis of progressive neurologic regression, often provoked by an infection, along with characteristic MRI findings (T2 prolongation in bilateral basal ganglia, in particular the putamen, brainstem nuclei, periaqueductal gray matter, and central white matter). Proton magnetic resonance spectroscopy may show increased lactate in the most affected areas. This description provides context for our study, because the increases in excreted urinary amino acids obtained with the ninhydrin-based Hitachi L-8800 analyzer are not typical of this disorder.

NONPHYSIOLOGICAL HYPERTRYPTOPHANURIA

True hypertryptophanuria can be caused either by a defect in the conversion of tryptophan to kynurenine or by an abnormality in renal amino acid transport. Although patients with Hartnup or Tada disease may exhibit a marked hypertryptophanuria, the degree to

QUESTIONS TO CONSIDER

1. What are 2 pathologic conditions that produce large increases in urine tryptophan?

2. What could potentially induce false increases in tryptophan concentrations when urine amino acids are quantified by ion-exchange liquid chromatographic separation and postcolumn ninhydrin derivatization before detection?

3. On the basis of their respective chemical structures, do any of the prescribed AEDs react with ninhydrin and thus interfere with tryptophan measurement?
which tryptophan was increased in this patient’s urine excretion suggested a nonphysiological mechanism. An inherent limitation of the Hitachi L-8800 amino acid system—and platforms based on liquid chromatography and spectrophotometry in general—is that analyte identification is based on retention times within the chromatographic system deployed. Thus, quantification may be compromised by interfering analytes coeluting with the analyte of interest. In our system, such a metabolite would both possess a tryptophan-like chromatographic affinity and react with ninhydrin. The tryptophan concentration of 71 μmol/g creatinine measured with the LC-MS/MS system was within the expected concentration interval for tryptophan and was more consistent with the patient’s clinical status. This fact, along with the enhanced specificity afforded by mass detection with LC-MS/MS analysis, confirmed our suspicion of pseudohypertryptophanuria in our patient.

POTENTIAL INFLUENCE OF ANTI EPILEPTIC DRUGS ON AMINO ACID CONCENTRATIONS

To identify the source of this artificial tryptophan increase, we considered the 5 prescribed therapeutic agents (Fig. 2) as potential interferences. The antiepileptic drug (AED) vigabatrin, a γ-vinyl analog of the neurotransmitter GABA, appeared the most likely interfering compound because of the presence of a free primary amine and a carboxylic acid within its chemical structure that ninhydrin might label analogously to amino acids. This drug, an irreversible inhibitor of the enzyme GABA aminotransferase (1) recently cleared by the US Food and Drug Administration, is used to treat spasms in infants and epileptic seizures. This induced reduction in GABA catabolism increases brain GABA concentrations to provide the drug’s antiepileptic effect, because GABA is an important inhibitory transmitter in the central nervous system. Vigabatrin is cleared primarily via renal excretion and does not undergo extensive metabolism upon administration. Furthermore, vigabatrin alters the amino acid concentrations in the urine of certain patients, with the induction of increases in GABA, β-alanine, and β-aminoisobutyric acid, as observed in our patient (1, 2).

To investigate this interference scenario, we qualitatively assessed the urinary presence of vigabatrin in house by an LC-MS/MS procedure that uses an Acquity UltraPerformance LC® System (Waters) coupled to a Waters Micromass® Quattro Premier MS/MS instrument. Amino acids were separated through ion-
pairing reversed-phase liquid chromatography, with the ion-pair reagent pentadecafluorooctanoic acid providing the enhanced separation selectivity. The analytical protocol followed the methodology of published strategies for underivatized amino acid analyses by LC-MS/MS (3–5). This analysis revealed a large vigabatrin peak in the optimized MS/MS chromatogram (precursor \( m/z \) > production ion \( m/z \): 130.1 \( m/z \) > 70.1 \( m/z \)). The identity of this parent drug was confirmed by a retention time identical to that of a pure vigabatrin solution. The chromatograms of several control urine samples analyzed by the identical procedure were devoid of metabolite peaks when they were examined with the vigabatrin-specific MS/MS transition.

The presumptive presence of this AED in the urine of our patient was further confirmed by adding increasing amounts of vigabatrin (i.e., 0, 500, 1500, and 7500 \( \mu \)mol/g creatinine) into aliquots of a control urine sample before UV analysis by the automated liquid chromatography amino acid analyzer. In the control urine sample, endogenous tryptophan eluted at 76.81 min. For the samples with added vigabatrin, the relative retention times were 76.86, 76.54, and 76.37 min for native tryptophan, and 77.82, 77.67, and 77.62 min for vigabatrin. The vigabatrin peak appeared immediately after the tryptophan peak in each of these samples, a result consistent with the 77.22-min elution time of the unknown interfering peak in our patient’s urine. This assignment is supported by the relative proximity of the elution time for the purported vigabatrin interferent to the structurally analogous GABA (i.e., 75.40 min). Of note is that although the AED concentrations tested in these in vitro experiments were not sufficiently high to completely obscure the tryptophan peak, it is clear that the potential for such a scenario would exist at higher in vivo urine concentrations. Additionally, the limited sample volume precluded quantitative assessment of GABA, \( \beta \)-alanine, and \( \beta \)-aminoisobutyric acid by LC-MS/MS. Thus, our assignment of the increases in these amino acids to vigabatrin therapy was based on the literature (1, 2).

This case highlights vigabatrin’s influence on urinary amino acid concentrations. In addition, it illustrates that knowledge of the ninhydrin-based chemical derivatization used within this amino acid analyzer provides the ability to distinguish potential interfering compounds according to their respective chemical structures and physicochemical properties. A careful review of both patient medications and the literature, especially with treatments that use re-
Clinical Case Study

POINT TO REMEMBER

- True hypertryptophanuria can be caused by a defect in the conversion of tryptophan to kynurenine (Hartnup disease) or by an abnormality in renal amino acid transport (e.g., Tada disease).
- Vigabatrin reacts with ninhydrin to interfere with urine amino acid quantification using a Hitachi L-8800 analyzer.
- Vigabatrin induces increases in urinary amino acids, particularly GABA, β-alanine, and β-aminoisobutyric acid.
- Knowledge of chemical structures and reaction mechanisms is critical for complete investigations of potentially interfering amino acid compounds.
- The literature describing potential interferences by therapeutic drugs should be consulted when non-physiological amino acid concentrations are obtained.

Recently approved therapeutics, should remain standard practice when laboratory results do not fit the patient’s clinical picture. Given the widespread use of this assay for metabolic screening, particularly in pediatric settings, these tenets should reduce the risk of false chromatogram interpretations. Finally, as demonstrated through the incremental value gained, a wider acceptance and implementation of rapidly emerging quantitative LC-MS/MS amino acid protocols (6–9) may lead to a reduction in misdiagnoses of rare metabolic disorders.

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 References


Commentary

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Ptolemy et al. illustrate the long-recognized problem of coeluting drug peaks in cation-exchange amino acid analysis with ninhydrin detection. Such interference is often noted in the urine of patients on antibiotic therapy (ampicillin, amoxicillin), as well as other medications and radiopaque dyes (diatrizoate meglumine). Although anticonvulsants are less often observed, vigabatrin interference in patient urine samples has previously been described by Preece et al. (1) as a large peak eluting near tryptophan (specifically between ammonia and ornithine), undoubtedly representing the same peak as that seen here. Such interference is traditionally evaluated by comparing the ratio of the spectrophoto-

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