CASE REPORT

Mitochondrial Carbonic Anhydrase VA Deficiency in Neonatal Hyperammonemic Encephalopathy: Case Report

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Abstract

Hyperammonemia can be a potentially fatal disorder, secondary to several different etiologies, most commonly urea cycle defects and organic acidurias. The deficiency of mitochondrial carbonic anhydrase VA, a recently recognized metabolic disorder, results from abnormalities in the *CA5A* gene. This gene plays an important role in ureagenesis and gluconeogenesis, resulting in a secondary deficiency of several carboxylases and presenting as neonatal hyperammonemic encephalopathy. We describe the case of an almost 5-year-old child who had neonatal encephalopathy secondary to hyperammonemia wherein carbonic anhydrase VA deficiency was identified in him. His growth and development are normal despite no diet or medication for several years. We report this case as fewer than 20 patients have been described in the literature.

Keywords: Brain Diseases, Metabolic, Inborn/etiology; Carbonic Anhydrases/deficiency; Carbonic Anhydrases/genetics; Infant, Newborn; Hyperammonemia/therapy; Hyperammonemia/etiology

Introduction

Carbonic anhydrases (CA) are zinc-containing metalloenzymes that catalyze the reversible hydration of carbon dioxide (Fig. 1). Carbonic anhydrases contribute to the regulation of acid-base balance, participating in a number of other physiological processes, such as carbon dioxide and bicarbonate transport, bone reabsorption, production of body fluids, gluconeogenesis, ureagenesis, and lipogenesis. At least 12 active carbonic anhydrase isoenzymes have emerged in humans and types VA and VB are located in mitochondria.[1-3] Carbonic anhydrase VA provides the formation of bicarbonate, a simple single carbon molecule that plays surprisingly important roles in diverse biological processes, being important to four hepatic mitochondrial enzymes involved in essential metabolic pathways: carbamoyl phosphate synthetase in urea cycle, pyruvate carboxylase (gluconeogenesis), propionyl-CoA carboxylase, and 3-methylcrotonyl-CoA carboxylase (branched chain amino acid catabolism) (Fig. 2).[4]

Ureagenesis appears to be the most sensitive pathway affected in carbonic anhydrases absence and, therefore, in CA-VA and CA-VB defects. The incapacity to provide enough bicarbonate for carbamoyl phosphate synthase 1 (CPS1) in the first step of the urea cycle results in hyperammonemia. As referred, gluconeogenesis is also impaired contributing to the catabolic status.[2]

The anhydrase type VA (CA-VA) deficiency (OMIM 615751) is a rare autosomal-recessive inborn error of metabolism first described in 2014 in four patients, from two different pedigrees.[5] It leads to a unique combination of biochemical findings: hyperammonemia, elevated lactate and ketone bodies, metabolic acidosis, hypoglycemia and excretion of carboxylase substrates.[5,6]

Case Report

We report the case of a 4-year-old boy born at term to unrelated Caucasian parents. He presented on day 2 of life with tachypnea, hypotonia, and tremor followed by encephalopathy secondary to hyperammonemia (311 µmol/L, reference values 18-72 µmol/L). Unlike

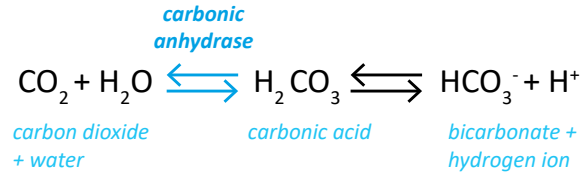

Figure 1. Role of carbonic anhydrase in the formation of bicarbonate. Carbonic anhydrase type VA is one of the mitochondrial isoforms of its enzyme.

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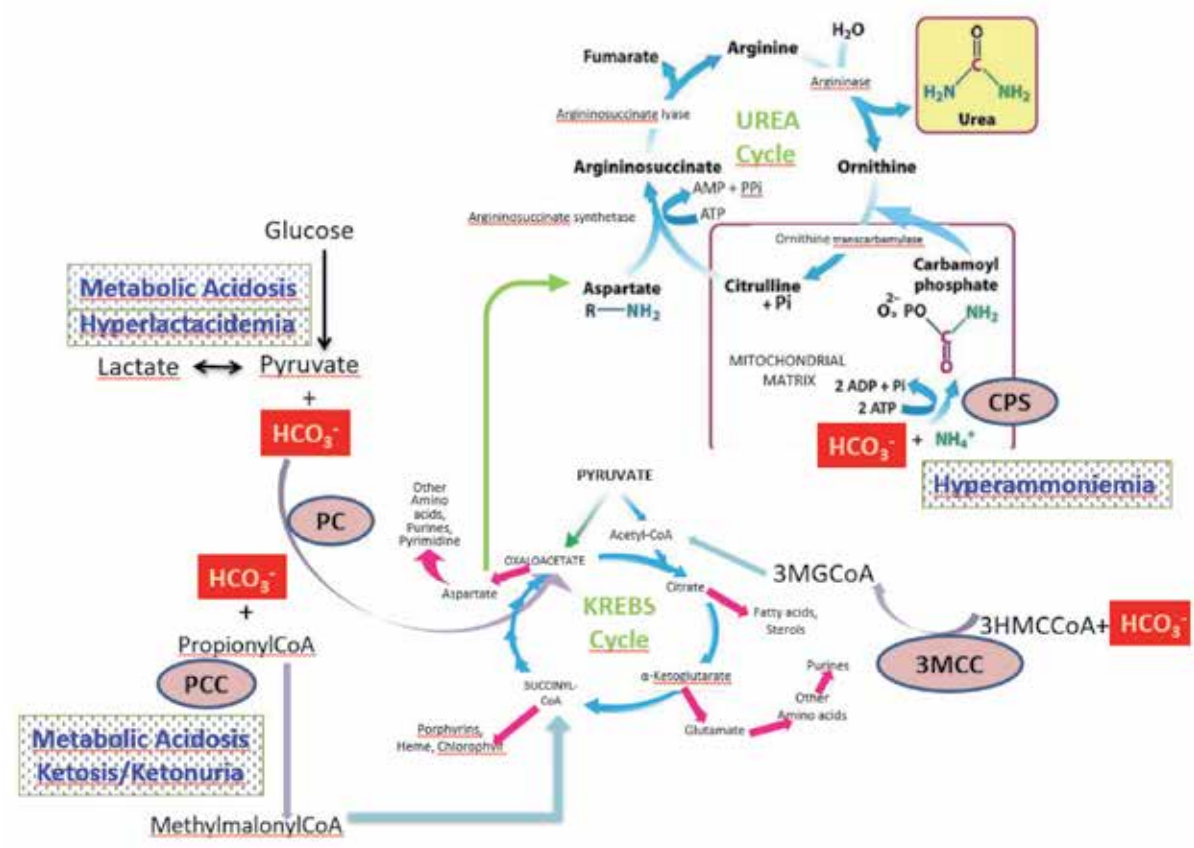

3MCC - 3-methylcrotonyl-CoA carboxylase; PC - pyruvate carboxylase; PCC - propionyl-CoA carboxylase; CPS - carbamoyl phosphate synthetase.
The enzymes involved in this defect are indicated in oval boxes.
Biochemical findings secondary to enzyme deficiencies are indicated in dotted rectangles.

Figure 2. Role of bicarbonate and, therefore, of carbonic anhydrase VA in the different pathways, together with their correlation.

what is described in urea cycle disorders, he presented persistent metabolic acidosis (pH 7.31, bicarbonate HCO_3^- 14 mmol/L, base excess -15.6), hyperlactacidemia (9.6 mmol/L and 6.5 mmol/L, reference values 0.5-2.2 mmol/L), transient hypoglycemia (minimum 2.1 mmol/L), and ketonuria (100 mg/dL).

Expanded neonatal screening of metabolic disorders was unremarkable. Plasma amino acids showed high glutamine (1255 µmol/L and 1127 µmol/L, reference values 243-822 µmol/L) and alanine (684 µmol/L and 1599 µmol/L, reference values 100-400 µmol/L) levels and normal/low citrulline (17.8 µmol/L and 9.0 µmol/L, reference values 15-30 µmol/L). Urinary organic acids chromatography revealed a high excretion of β-hydroxybutyric and acetoacetic acids and the orotic acid level was normal.

Molecular studies of the N-acetylglutamate synthase (*NAGS*) and *CPS1* genes showed no pathogenic mutations. As CA-VA deficiency was later suspected, molecular studies of the *CA5A* gene were conducted and revealed a c.774G>C (p.Gln258His) splicing heterozygous mutation in exon 6 and a large heterozygous deletion c.(142+1_143-1)_(459+1_460-1)del p.? of exons 2 and 3, confirming the diagnosis of this disorder.

To the best of our knowledge, the described mutation obtained by multiplex ligation-dependent probe amplification has not been previously described but as it affects the last nucleotide of exon 6, the donor splice-site gets destroyed, most probably leading to alternative splicing. In addition, the deletion that the index patient presented has not been described previously and further studies by low range polymerase chain reaction (LR-PCR) could not characterize the exact point of the deletion. The parents and an uncle, studied for genetic counseling and an eventual pre-natal diagnosis, are heterozygous for the defects found in the index case.

The patient was treated with mechanical ventilation, protein free formula, intravenous dextrose, sodium benzoate, phenylbutyrate, and L-arginine showing the transient improvement of ammonia levels. On day 4, as he presented no significant clinical amelioration and further increase of the ammonia level, carglumic acid was added despite no etiology at that point, after which the patient's symptoms resolved completely and ammonia normalized swiftly (Fig. 3).

He was kept on a low protein diet as well as sodium

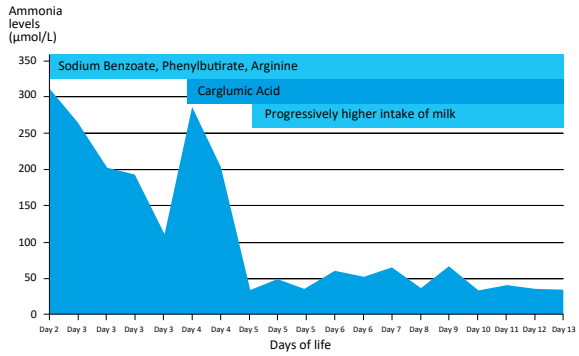

Figure 3. Progression of plasma ammonia levels (µmol/L) and therapy over the first two weeks of life.

benzoate and phenylbutyrate and arginine for several months but, after non-adjustment of the medication to body weight, as the patient was well and the ammonia levels were normal, these were suspended at 6 months of age.

Follow-up at 4.5 years shows normal growth with weight 19.1 kg (percentile 90) and height 105.9 cm (percentile 50), no hepatomegaly and a normal neurological examination as well as an optimal psychomotor development and speech (recognizes numbers and letters, knows how to write his name using capital letters). He has also had no decompensations and has had normal ammonia levels even though he has not been on any medication or diet since the age of six months, despite some minor intercurrent illnesses.

Discussion

Hyperammonemia in a previously apparently healthy newborn is usually secondary to an inherited error of metabolism and mainly related to urea cycle defects or organic acidurias, although other defects causing neonatal hyperammonemia have also been described in the past few years.[7,8]

Despite high levels of glutamine levels in plasma, this patient presented no respiratory alkalosis as is typical in urea cycle defects, and a normal orotic acid in the urine. On the other hand normal expanded neonatal screening and organic acids also exclude known organic acidurias. The organic acids in the urine of this patient presented ketonuria with high levels of beta-hydroxybutyrate and acetoacetate levels, but unlike what is described in some patients with CA-VA deficiency, no carboxylase substrates or related metabolites, such as 3-OH-propionate, propionylglycine, methylcitrate, or lactate. On the other hand, while newborn screening using tandem mass spectrometry (MS/MS) can theoretically also detect carboxylase substrates (specifically C3 and C5OH levels as seen in multiple carboxylase deficiency), they were unremarkable in the first four individuals reported as in this patient.[5]

However, metabolic acidosis, hyperlactacidemia, transient hypoglycemia, and ketonuria as well as high levels of glutamine and alanine pointed to the possibility of CA-VA deficiency that was confirmed by genetic studies.[5]

As only a few cases of a defect *CA5A* gene have been described, we underline that CA-VA deficiency, a potentially treatable condition, should be excluded in neonatal hyperammonemia, especially if it is associated with lactic acidosis, hypoglycemia, and ketonuria.

Finding the correct etiology of hyperammonemia can be a challenge, but it may be essential for providing the correct treatment. Traditionally, the treatment of hyperammonemia in patients includes dietary protein restriction to reduce nitrogen flux and the administration of ammonia scavenger medications, such as benzoate, phenylacetate, and phenylbutyrate, to divert excess nitrogen into a nontoxic, excretable metabolite pool as well as different extracorporeal procedures in very severe cases for toxin removal. The treatment of organic acidurias also includes vitamins acting as cofactors and carnitine that promotes the urinary excretion of carnitine bound organic acids.[9,10]

Carglumic acid (short name for N-carbamoyl-L-glutamic acid), as a synthetic analogue of N-acetyl glutamate (NAG), is an orphan drug initially approved for the acute and long-term management of hyperammonemia caused by N-acetyl glutamate synthase deficiency and later has also been used in other urea cycle defects as well as to maintain normal ammonia levels in organic acidemia.[11] In the patient we describe treatment with intravenous dextrose, a protein free formula, sodium benzoate and phenylbutyrate, and L-arginine showed a transient improvement but symptoms resolved completely and ammonia normalized once carglumic acid was introduced, showing that this drug is highly effective for this disorder. The mechanism of action in CA-VA deficiency is acting as a CPS1 cofactor enhancing ureagenesis and activating the urea cycle.

Children with carbonic anhydrase VA deficiency usually first experience episodes of the disorder by the age of 2. These episodes may be triggered by fasting for longer than usual between meals or when energy demands are increased, such as during illness. Between episodes, children with carbonic anhydrase VA deficiency are generally healthy, and more than half have no further episodes after the first one. Some affected children have mildly delayed development or learning disabilities, while others develop normally for their age.[4,5]

We report this patient because CA-VA deficiency is a recently discovered inborn error of metabolism and only a few cases have been described. This disorder should be excluded in newborns with hyperammonemia especially if patients present with metabolic acidosis. We emphasize the therapeutic potential of carglumic acid as an additional and highly effective treatment of encephalopathy induced by conditions such as CA-VA deficiency. The fact that the patient has had no further decompensations, has normal ammonia levels, and is growing well as well as in an almost five year old follow-up shows an optimal development despite no treatment also points to this being a transient and benign disorder. It is also possible that some cases described in the past as transient hyperammonemia of the newborn may in fact be secondary to a CA-VA defect. The optimal evolution of most of the described cases can also represent great hope for some parents regarding the prognosis of neonatal hyperammonemia.

WHAT THIS CASE REPORT ADDS

- In all children with decreased consciousness/coma, ammonia levels should be ascertained.
- Hyperammonemia can be secondary to several different etiologies, most commonly urea cycle defects and organic acidurias, but other rarer or even unknown diseases should not be excluded.
- Careful evaluation of the laboratory parameters may help in finding the etiology of hyperammonemia.
- Neonatal hyperammonemia can be a potentially fatal disorder, but if it is appropriately treated in the acute phase, it may have a very good prognosis.

Conflicts of Interest

The authors declare that there were no conflicts of interest in conducting this work.

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Consent for publication

Consent for publication was obtained.

Confidentiality of data

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

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Deficiência de Anidrase Carbónica Mitocondrial VA na Encefalopatia Hiperamonémica Neonatal: Caso Clínico

Resumo

A hiperamonemia pode ser um distúrbio potencialmente fatal, secundário a várias etiologias diferentes, mais comummente defeitos do ciclo da ureia e acidúrias orgânicas. A deficiência de anidrase carbónica mitocondrial VA, um distúrbio metabólico recentemente reconhecido, resulta de alterações no gene *CA5A*. Esse gene desempenha um papel importante na ureogénese e gliconeogénese, resultando em deficiência secundária de várias carboxilases e apresentando-se como encefalopatia hiperamonémica neonatal. Descrevemos o caso clínico de uma criança com quase 5 anos de idade que apresentou encefalopatia neonatal secundária a hiperamonemia, resultante de deficiência de anidrase carbónica mitocondrial VA. Enfatizamos o caso de a criança ter crescimento e desenvolvimento normais, apesar de nenhuma dieta ou medicamento por vários anos. Relatamos este caso pela sua raridade, com menos de 20 pacientes descritos na literatura.

Palavras-Chave: Anidrases Carbónicas/deficiência; Anidrases Carbónicas/genética; Encefalopatias Metabólicas Congénitas/etiologia; Hiperamonemia/etiologia; Hiperamonemia/tratamento; Recém-Nascido