REVIEW

PHARMACOTHERAPEUTICAL CONSIDERATIONS IN THE TREATMENT AND MANAGEMENT OF NEONATAL HYPERAMMONAEMIA

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Abstract

Amoniemia is a normal biochemical condition, the result of nitrogen compounds metabolism. The normal level of free ammonia in human plasma is usually less than 35 μmol/L. Increased ammonia levels over 35 μmol/L is named hyperammonaemia (HA), a metabolic condition that becomes clinically evident by disrupting the neurotransmitters systems caused by neuronal damages. Neonatal hyperammonaemia is rare, being caused in most of the cases by the inborn error of the metabolism due to the urea cycle enzymes deficiency. They are pathological conditions with vital concern, whose recognition, early diagnosis and treatment are essential for the survival and the quality of life. The main treatment is medication together with a dietary approach.

Rezumat

Amoniemia este o stare biochimică normală, rezultat al metabolismului compuşilor azotaţi. Valorile normale ale amoniului liber in plasma umană este de regulă sub 35 μmol/L. Creşterea amoniemiei peste 35 μmol/L constituie hiperamoniemia (HA), o stare metabolică ce devine manifestă clinic urmare, în principal, afectării funcţiei normale a celulei neuronale. Hiperamoniemiile neonatale sunt rare, fiind cauzate, în cele mai multe cazuri, de erori metabolice congenitale datorate unor deficite ale sistemelor enzimatice din ciclul ureei. HA sunt stări patologice cu ameninţare vitală, a căror recunoaştere, diagnostic şi tratament precoce sunt esenţiale pentru supravieţuire şi calitatea vieţii. Tratamentul principal este medicamentos, fiind însoţit de o abordare nutriţională adecvată.

Keywords: hyperammonaemia, urea, inborn errors of metabolism

Introduction

Hyperammonaemia is a metabolic condition characterized by elevated levels of ammonia in the blood over 35 μmol/L. HA is an etiological key factor in urea cycle disorders (UCD) and plays a major role in central nervous system (CNS) toxicity. The toxic effects of ammonia on the CNS are more severe in the developing brain than in the adult brain. It becomes clinically manifested mainly as the consequence of impaired neurotransmitter system that will affect normal neuronal function. Ammoniemia is a normal biochemical condition. Ammonia is produced primarily in the bowel by bacterial hydrolysis of urea and other nitrogen compounds; it also arises from other metabolic processes such as cycle purines transamination of amino acids in the skeletal muscles. The conditions in which most often occurs the HA are congenital deficiencies of urea cycle enzymes, liver failure, Reye's syndrome. The incidence of urea cycle disorders in the population is considered to be at least 1 in 35000 births.

UCD are resulting from defects in any one of the six enzymes and 2 transporters involved in the hepatic removal of ammonia from the bloodstream by conversion to urea which is excreted by the kidneys.

The liver synthesis of urea follows several steps

First, the synthesis of carbamoyl phosphate from ammonia and CO_2 occurs, in the presence of ATP and under the control of carbamoyl phosphate synthetase (CPS1), enzyme with exclusively hepatic distribution and activated acetylglutamate. It follows the condensation of carbamoyl phosphate with ornithine to form citrulline under the action of ornithine-carbamoyl transferase (OTC). Then, citrulline is combined with aspartate to form arginine-succinic acid under the action of argininosuccinate-synthetase (ASS). Argininosuccinic acid is cleaved by arginino-succinate lyase (ASL) in fumarate and arginine. In the last step, arginine is

hydrolysed by arginase (ARG), releasing urea molecule, with ornithine regeneration.

The severe deficiency or absent activity of any enzyme involved in the urea cycle (N-acetylglutamate synthetase (NAGS), CPS1, OTC, ASS, ASL) or cofactor (N-acetylglutamate) leads to the accumulation of ammonia and other metabolite precursors in the blood during the first few days of life. Thus, for the six enzymatic stages, there were described the following conditions: N-acetylglutamate synthetase deficiency (NAGS), carbamoyl phosphate synthetase deficiency (CPS), ornithine transcarbamylase deficiency (OTC), arginosuccinate synthetase deficiency (citrullinemia), arginosuccinate lyase (arginosuccinic aciduria), arginase deficiency (argininemia).

N-acetylglutamate synthetase deficiency (Online Mendelian Inheritance in Man (OMIM) 237310)

N-acetylglutamate synthetase deficiency (NAGS) is an autosomal recessive disorder. Mutations appearing in the NAGS gene located on chromosome 17 (17q21) determine a total or partial absence of NAGS activity. The majority of patients are homozygous or compound heterozygous. The absence of NAGS enzyme determines an excessive accumulation of nitrogen, in the form of ammonia, in the blood. Excess ammonia, which is neurotoxic, travels to the central nervous system through the blood, resulting in the symptoms and physical findings of NAGS deficiency. In most cases, the onset of symptoms occurs at, or shortly following birth. Symptoms include vomiting, refusal to eat, progressive lethargy and coma.

Specifically, the NAGS enzyme is an activator of another enzyme of the urea cycle known as carbamoyl phosphate synthetase (CPS1), so the clinical and bio-chemical features of the disorder are indistinguishable from CPS1 deficiency [12, 32].

Carbamoyl phosphate synthetase deficiency (OMIM 237300)

Carbamoyl phosphate synthetase deficiency is an autosomal recessive disorder, caused by mutations in the CPS1 gene, located on chromosome 2 (2q34). The disorder is relatively rare; in Japan the incidence is estimated at 1/800000 new-borns. The CPS1 gene provides instructions for making the enzyme carbamoyl phosphate synthetase1. The specific role of the CPS enzyme is to control the first step of the urea cycle, a reaction in which excess nitrogen compounds are incorporated into the cycle to be processed. Neonates develop lethargy, hypothermia, vomiting and irritability. The HA is severe as the patients with partial enzyme deficiency present a relapsing syndrome of lethargy and irritability upon exposure to protein. Brain damage can occur in both neonatal and late-onset groups [19].

Ornithine transcarbamoylase deficiency (OMIM 311250)

Ornithine transcarbamoylase deficiency is an X-linked genetic condition; the OTC gene is located on chromosome X (Xp21.4) and contains ten exons and nine introns which codify a protein of 322 amino acids expressed in the liver and the intestinal mucosa [10]. There are approximately 417 disease-causing mutations in the OTC gene [15]. The hemizygous males are the most frequent affected meanwhile the females are usually healthy, but carriers (females have two X chromosomes and only one carries the defective gene). About 20% of female carriers may present some neurocognitive deficiency depending on lyonization patterns. The OTC gene provides instructions for making the ornithine transcarbamoylase enzyme. OTC deficiency is the most common disorder of urea cycle disorders, accounting for nearly 50% of all cases. The estimated frequency is 1/50000 - 80000 people. The symptoms may become evident at any age and the most severe form occurs in the first few days of life. The symptoms are vomiting, progressive lethargy and irritability, hepatomegaly, unwilling to eat, hypotonia ("floppy" baby) seizures or coma. Without treatment, infants with severe form may develop coma or neurological abnormalities such as mental disability, developmental delays and cerebral palsy. Sometimes the symptoms do not appear until later in life. People with late-onset may develop headaches, vomiting, nausea, dysarthria, ataxia, confusion, hallucinations and blurred vision [39].

Arginosuccinate synthetase deficiency (OMIM 215700)

Arginosuccinate synthetase deficiency (citrullinemia) is an autosomal recessive disease; the ASS gene is located on chromosome 9 (9q34) [38]. At least 50 mutations that cause type I citrullinemia have been identified in the ASS gene. These mutations affect the structure of the enzyme and its ability to bind to citrulline, aspartate, and other substrates. A few mutations lead to the production of an abnormal short enzyme that cannot effectively play its role in the urea cycle.

Affected new-borns have a complete absence of enzyme activity. Milder forms with later onset have preservation of some enzyme activity. New-borns appear normal at birth but then develop hyper-ammonemia, progressive lethargy, feeding disturbances, vomiting. Some of them are developing signs of increased intracranial pressure which is associated with spasticity and seizures. Even with prompt treatment, survivors often show severe neurological deficiencies.

Arginosuccinate lyase deficiency (OMIM 207900)

Arginosuccinate lyase deficiency affects the fourth step in the urea cycle, in which argininosuccinic acid is cleaved to produce arginine and fumarate. ASL deficiency is inherited in an autosomal recessive manner. The ASL gene is located on chromosome 7 (7q11.21). This gene encodes a member of the lyase 1 family. The encoded protein forms a cytosolic homo-tetramer and primarily catalyses the reversible hydrolytic cleavage of argininosuccinate into arginine and

fumarate, an essential liver step in detoxifying ammonia *via* the urea cycle [4].

ASL deficiency is rare, the incidence being estimated in United States to 1 in 70000 live births. In Finland 20 cases of ASA lyase deficiency had been reported by 2007 [9]. In Romania there is only one child with ASL deficiency reported and treated in the Regional Center of Medical Genetics, Oradea, Romania.

The symptoms are variable. There are two forms: severe neonatal onset form and a late onset form. The severe neonatal onset form is characterized by HA within the first few days after birth associated with vomiting, lethargy, hypothermia and poor feeding. In the absence of treatment lethargy, seizures and coma develop, resulting in death. The form with late onset ranges from episodic HA (triggered by acute infection or stress) to cognitive impairment, behavioural abnormalities, learning disabilities in the absence of any documented episodes of HA [7, 10]. Manifestations of ASL deficiency that appear to be unrelated to the severity or duration of hyperammonemic episodes include: neurocognitive deficiencies, developmental delay, seizures and learning disability, liver diseases: hepatitis, cirrhosis [22, 24, 33]. About half of individuals with ASL deficiency have a coarse hair that breaks easily, surrounded by areas of partial alopecia [16].

Arginase deficiency (OMIM 207800)

Arginase deficiency (argininemia) is a recessive autosomal disorder in which the conversion of arginine to urea and ornithine is blocked resulting in progressive spastic tetraplegia, especially in the lower extremities. Arginase 1-gene (ARG1) is located on chromosome 6, long arm (6q23.2). This gene encodes an enzyme (arginase) that catalyses arginine hydrolysis to ornithine and urea. Mutations in the ARG1 gene result in the production of an abnormal arginase isoform. Prevalence is estimated at 1/300000 - 1000000 people. Unlike patients with other urea cycle defects, patients with argininemia rarely exhibit hyperammonemic encephalopathy. The onset of argininemia is typically insidious, with spastic paraplegia (the most obvious sign), mental retardation, failure to thrive and seizures - arising during childhood. Other symptoms may include the loss of developmental milestones, intellectual disability, tremor and ataxia. Patients with argininemia show a failure to thrive and a persistent low growth rate that leads to a short stature. Carvalho and Prasad reported that around 81% of patients with argininemia developed growth restriction [14, 27].

Diagnosis of urea cycle disorders

Laboratory investigations: HA with respiratory alkalosis is the classical finding seen during periods of metabolic decompensation in all urea cycle disorders.

Enzyme tests tend to be removed from the test battery of these conditions, replaced by genetic tests. However, when genetic tests cannot be performed or when they are negative, enzymatic assays are indicated. Thus, hepatic biopsy can identify the deficiencies of CPS, NAGS and OTC; from erythrocytes the arginine deficiency can be identified; from skin fibroblasts is determined ASAS, ASL and HHH; intestinal mucosa can cause CPS and OTC.

Genetic tests give accurate diagnostics, mutation analysis being possible for all the enzymes involved in the urea cycle. Molecular diagnosis may be helpful when the biochemical findings are equivocal. Mutations in the corresponding genes have been identified in patients of all urea cycle disorders. Mutation detection has at least 80% sensitivity [35, 36] and allows carrier identification, prenatal diagnosis, facilitating pedigree analysis, genetic counselling and in some cases genotype-phenotype correlations [17, 32].

New-born screening (NBS). Urea cycle disorders patients manifesting severe neonatal HA benefit of new-borns screening, because of their poor prognosis, although the family would benefit from knowing the earlier diagnosis [3]. However, NAGS, CPS1 and OCD are generally not screened for, given the instability of glutamine and the low specificity and sensitivity for detection of decreases in the citrulline level.

Prenatal diagnosis is also possible, either by molecular tests (DNA genetic testing) obtained from chorionic villus or cells in amniotic fluid, or by analysis of enzymatic activity in amniotic cells, chorionic villus, liver cells (foetal liver biopsy puncture) or foetal erythrocytes.

Treatment

The purposes of the treatment are to correct the altered biochemical findings and to provide an appropriate nutritional status of child's development. From a practical standpoint, the management of HA is directed to different evolutionary stages of clinical manifestations and aims: the emergency treatment of the seizures and coma (at onset and during recurrent episodes of HA), chronic treatment (inter-critical), surgery, preventive treatment and genetic counselling [5, 10].

Treatment of seizures and HA coma

The treatment starts immediate after confirming the relation between clinical signs and HA, by dosing ammonia in the blood. The purpose of the treatment is mainly aimed to the immediate cessation of seizures and to reduce levels of ammonia. Anticonvulsant therapy includes intravenous diazepam, phenobarbital, phenytoin. Valproic acid should not be used to treat seizures because it decreases urea cycle function and increases blood levels of ammonia [28]. It is not recommended to use corticosteroids (they cause a negative nitrogen balance) and mannitol (not useful in the treatment of cerebral oedema induced by HA).

The threshold value from which imminent coma appears in HA is 150 - 200 μmol/L. HA treatment is done on the one hand, by the immediate cessation

protein intake, and on the other hand, as soon as possible, by removing the excess ammonia [1]. The method of choice is haemodialysis. As alternative methods may be used peritoneal dialysis, exchange transfusion or continuous arterial-venous hemofiltration [8].

Sodium benzoate and sodium phenylbutyrate act as alternative ways to increase the urinary nitrogen excretion. It is estimated that for every gram of sodium phenylbutyrate administered, it is excreted between 0.12 g and 0.15 g of nitrogen as phenylacetylglutamine. Arginine is useful only for the treatment of CPS, OTC, ASS and ASL deficiency [21, 30].

The first step is to stop the protein intake and to ensure intravenously substances which increase caloric intake: solution of dextrose 10 - 15%, lipid emulsion infusion (1 g lipids/kg/24 hours) then therapy with sodium benzoate and sodium phenylbutyrate should be initiated. There are used for the first administration: sodium benzoate 250 mg/kg and sodium phenylbutyrate 250 mg/kg, arginine hydrochloride 200 - 800 mg/kg. These three drugs are administered together i.v., in 10% dextrose solution (total 20 mL/kg) over 1 - 2 hours. The dose is then administered by continuous infusion over 24 hours, in equal doses to the initial one. We note that for the first two substances the dose can be doubled (250 - 500 mg/kg/24 hours). There is currently a concentrate, sterile and aqueous solution containing sodium phenylacetate and sodium benzoate 10%/10%. Sodium phenylacetate is a crystalline powder of white colour with strong odour, soluble in water. Sodium benzoate is a white powder, odourless and soluble in water. The drug is a concentrated solution and must be diluted before administration to the central venous catheter with 10% dextrose solution.

Peripheral venous administration is not recommended because it causes burning sensation. Initially it is administered a loading dose during 90 - 120 minutes followed by a continuous intravenous infusion for 24 hours. Intravenous infusion is maintained until the ammonia levels are normalized and patients resume their oral tolerance. Nausea and vomiting can occur during administration, therefore it is recommended to also administrate an antiemetic. The clinical status (breathing, peripheral pulse, blood pressure, back of the eye) as well as ammoniemia, glutamine, plasma levels of amino acids, glucose, electrolytes, Astrup parameters (a method for blood acid - base disorders), SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase) are monitored [13].

Arginine hydrochloride is administered together with sodium phenylacetate and sodium benzoate. Administration of arginine is useful for all of HA except arginine deficiency. It is administered in doses of 6 mL/kg for 90 minutes, then as a continuous intravenous infusion for 24 hours. Hyperchloraemic acidosis can occur, therefore blood acid-base parameters should be monitored and, if required, administration of sodium bicarbonate can be recommended.

L-carnitine is an essential cofactor in the metabolism of long-chain fatty acids. It is synthesized in the liver and kidneys from lysine and methionine. Carnitine action against ammonia toxicity is due to the protective effect against neurotoxicity caused by glutamate [6, 21]. Carnitine is presented in ampoules of 200 mg/mL, and is administered in a loading dose of 100 mg/kg over 30 min, then as a maintenance dose of 15 mg/kg body, every 4 hours during 10 to 30 min.

The intake of these medicines is followed by the ammonia level determination at 8 hours intervals. In the absence of a favourable response, haemodialysis or peritoneal dialysis are practiced (the level of ammonaemia normalizes within 24 - 48 hours).

The amount of ammonia formed in the intestines is reduced by low protein intake of 0.5 - 1 g/kg/24 hours. If the patient's condition has improved, neomycin 50 mg/kg and lactulose are associated to reduce ammonia forming intestinal flora. When the ammonia levels return to normal values, treatment is switched to oral administration.

Chronic Treatment (inter-critical)

The majority of the HA have a chronic evolution, with episodic exacerbations. The goal of the treatment is to prevent acute episodes and to limit their recurrence. Maintaining a balance of metabolic ammonia levels can be achieved by two means: protein - restricted diet and specific medication to lower ammonia levels.

The dietary approach should respect the following principles: low protein intake (0.7 g/kg/day or 0.7 g protein/kg/day of essential aminoacids in mixture, the usual protein intake in infants under 6 months is 1.5 - 2 g/kg/day); additional arginine, 200 - 400 mg/kg/24 hours; providing enough calories for energy needs; gastric gavage or gastrostomy are the most efficient ways to administer fluids, food and medicines for oral use.

HA specific drug therapy

Sodium benzoate is an active metabolite agent: in a first phase it is conjugated with coenzyme A to form benzoyl CoA which is then conjugated with glycine in the mitochondria of the liver and kidneys to form hippurate which is rapidly excreted by the kidneys by glomerular filtration and tubular secretion. One mole of hippurate contains one mole of nitrogen, so effective hippurate is an alternative way of removing excess nitrogen [13]. The sodium benzoate is recommended in doses of 250 - 500 mg/kg/24 hours, in three doses.

Sodium phenylbutyrate reduces elevated plasma ammonia and glutamine levels in patients with urea cycle disorders. The daily doses administered to each

patient must be adjusted, depending on protein tolerance and the daily dietary protein intake required for growth and development. In children with the weight less than 20 kg, the daily dose is 450 - 600 mg/kg/day, and for children weighing more than 20 kg, adolescents and adults, the recommended dose is 9.9 - 13.0 g/m^2/day.

Sodium phenylbutyrate is a prodrug rapidly metabolized in phenylacetate. It conjugates with glutamine *via* acetylation to form phenylacetylglutamine which is then excreted by the kidneys. Esterases also hydrolysed sodium phenylacetate in liver and blood. For each gram of sodium phenylbutyrate administered, there are eliminated 0.12 and 0.15 g nitrogen as phenylacetyl-glutamine. The mean time to peak concentration is 3.74 hours for sodium phenyl-butyrate and 3.43 hours for sodium phenylacetate and the mean maximum concentration are 48.5 mg/mL and 68.5 mg/mL, respectively. Elimination half-times are 1.2 hours and 2.4 hours respectively [2].

Sodium benzoate and sodium phenylbutyrate must be used with caution in infants with hyperbilirubinemia because bilirubin may enhance its action after being released from the albumin.

L-carnitine is administered orally 100 mg/kg/day, every 6 hours as a solution (1 g/10 mL) or as 330 mg tablets [25].

Arginine is administered associated with phenylacetate or benzoate or both, to avoid catabolic states favouring the installation of hyperammonemia.

The intestinal absorption of ammonia can be reduced by administration of broad-spectrum oral antibiotics (e.g. neomycin) and lactulose, because acidification of the intestine reduces absorption.

Undercurrent infections require appropriate treatment, as they are hyper catabolic states likely to trigger the occurrence of acute clinical-biological manifestations.

Surgical Treatment

In patients with severe liver disease the liver transplantation is the method of choice to prevent the HA crisis. Neurological dysfunction and hepatic destruction are factors that play a major role in the decision for transplantation [31].

Liver transplantation is recommended in all types of UCD except N-acetylglutamate synthase deficiency (NAGSD) and the (hyperornithinemia – hyper-ammonemia - homocitrullinuria) HHH syndrome. The transplant is curative as far as enzyme deficiencies are concerned and allows cessation of the low-protein diet and regular alternative pathway therapy, but the neurological sequels are not influenced [37]. Transplanted patients require immunological therapy and long-term follow-up.

The inability to synthesize arginine extrahepatically persists, but this metabolic aberration has no recognized clinical impact. Thus, liver transplantation compared to drug therapy in UCD patients is the best alternative for the quality of life.

Preventive treatment

Primary prevention is to avoid new cases by competent genetic counselling in affected families. Long term secondary prevention is aimed to prevent episodes of HA by the protein restriction, special formulas and the use of oral medications to eliminate ammonia. It is recommended to avoid the risk factors (respiratory and gastrointestinal diseases, immunizations), the administration of vitamins and fluoride supplements and the correct use of anti-pyretic drugs. HA defects have a lifelong persistence. They generally have a moderate evolution of the acute episodes that can occur under the influence of the mentioned risk factors [18, 22].

Medical supervision, care and recovery of these children raise serious problems for the family and society through their addiction, by the high costs of care, treatment and/or recovery. The largest part of these costs could be avoided by the early diagnosis and treatment as well as rigorous monitoring of the progress.

Genetic counselling. CPS1, ASS1, ASL, NAGS and ARG deficiency, are transmitted (autosomal recessive) AR, with 25% recurrence risk [12]. Sibling of an affected individual has a 2/3 risk of being a carrier. OTC deficiency is transmitted by X-linked recessive. Boys are the most affected children of carrier mothers and healthy fathers. Affected men will have all daughters carriers [13, 19].

Conclusions

HA are diseases of vital concern, whose recognition, early diagnosis and treatment are essential for the survival and the quality of life. They are presented with a great diversity of aetiology, clinical, biological and evolutive features. HA is a medical emergency requiring intensive and complex treatment. Dietary and drug effectiveness are well-known, therefore any effort in early diagnosis/treatment or long-term monitoring is not enough high.

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