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Transition to glycerol phenylbutyrate for the management of urea cycle disorders: clinical experiences

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Abstract. – BACKGROUND: Urea cycle disorders (UCDs) are a group of rare inborn diseases caused by a deficiency in one of the six enzymes or one of the two transporters involved in the urea cycle. The most common biochemical feature is elevated blood ammonia levels, which can be toxic at high levels, especially to the brain and may manifest as encephalopathy if left untreated. Glycerol phenylbutyrate (GPB) is currently approved for use in the USA and Europe for patients of all ages with UCD who cannot be managed with protein restriction and/or amino acid supplementation alone. This article presents the author's experience in different exemplary settings and depicts the most efficient management of UCDs with GPB.

CASE PRESENTATION: Six patient histories are described. 4 had OCT, one citrullinemia, and one argininosuccinic aciduria. Treatment with GPB was started between 2 days and 14 years of age. Before GPB, one patient had not been treated, 4 had received sodium phenylbutyrate (NaPB), and one Na benzoate.

CONCLUSIONS: Overall, treatment with GPB was followed by a relevant metabolic improvement, resulting in better therapeutic compliance, reduced hospitalization, and improved quality of life.

Key Words:
Urea cycle disorder, Glycerol phenylbutyrate, Quality of life, Adherence.

Background

Urea cycle disorders (UCDs) are rare inborn diseases associated with severe neurological and mental impairment if not timely treated. This article presents the authors' experience in different exemplary settings and depicts the most efficient management of UCDs with GPB. The history of 6 children who switched from other nitrogen-scavenging drugs to GPB is described (Table I), reporting biochemical data from all the 12 patients presently on GPB (Table II).

Urea Cycle Disorders

UCDs are a group of rare inborn diseases caused by a deficiency in one of the six enzymes or one of the two transporters involved in the urea cycle. The overall incidence is 1 in 35,000 and, within the group, ornithine transcarbamylase deficiency (OTCD) is the most common one (~1 in 56,500) while the least common conditions have incidences less than 1 in 2,000,000[1]. Except for OTCD, which is caused by X-Linked mutation on the OTC gene, all the UCDs are transmitted as autosomal recessive traits[2]. The biochemical most common feature is the elevated plasmatic value of ammonia, which can be toxic at high levels, especially to the brain, and may manifest as encephalopathy if left untreated[3]. UCDs can manifest at any age, depending on the defect type and the enzymatic deficiency's severity. A complete defect usually presents as neonatal hyperammonemic encephalopathy, which can rapidly lead to death (reported between 19% and 60%) or severe neurological and mental impairment[3]. Although timely treatment is necessary to prevent death or serious damage, critically ill infants are frequently misdiagnosed if newborn screening tests are not routinely performed, as early symptoms of UCDs may be non-specific[3]. A partial defect may present later in life, often after a precipitating event, such as excess protein intake, infection, trauma, surgery, or deliverance[4]. It is noteworthy that all UCD patients are at risk of hyperammonemia, regardless of the level of enzymatic deficiency. Acute hyperammonemia symptoms include somnolence, lethargy, seizures, vomit-

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Table I. Clinical and biochemical data of patients describd in this report.

Patient n	Diagnosis	Genetic analysis	Switched from	Age at GPB start	Dose GPB	Associated drugs	Notes
1	OCT	Gene OTC: het c.717G>A	Naïve	2 days	0.2 ml × 4 or 250 mg/kg or 3.2 ml/m^2	Citrulline	At switch to GPB: Glutamine 535 µmol/l PT 54% INR 1.54
2	Citrullinemia	Gene ASS: het c.814C>T/ IVS5+1G>A	NaPB	3.5 years	0.7 ml × 3 or 200 mg/kg or 4.2 ml/m^2	Arginine, NaPB	Three hospitalizations for metabolic failure (one with septic shock) before switch; no hospitalizations from switch to 7 years old
3	OCT	Gene OTC: het c.422G>A	NaPB	14 years	3 ml × 3 or 180 mg/kg or 6 ml/mq	Citrulline, Na benzoate	At switch to GPB: Glutamine 951 µmol/L
4	Argininosuccinic aciduria	Gene ASL: het c.638G>A/ c.649C>T	Na benzoate	13.5 years	2.7 ml × 4 or 7.2 ml/mq or 216 mg/kg	Arginine	At switch to GPB: Glutamine 855 µmo/L SGPT 46 IU/l
5	OCT	–	NaPB	5.5 years	1 ml × 3 or 206 mg/kg or 4 ml/mq	Arginine, Na benzoate	At switch to GPB: Glutamine 1065 µmo/L Orotic acid 7.97 mmol/mol creatinine. Day-hospital was attended over 10-times/ year before, and 2-times/year after switch to GPB
6	OCT	Gene OTC: het c.626C>T	NaPB	2.5 years	1 ml × 3 or 220 mg/kg or 5 ml/mq	Arginine/ Na benzoate/ citrulline	At switch to GPB: Glutamine 994 µmo/L Orotic acid 156 mmol/mol creatinine

Table II. Clinical and biochemical data of patients not described here, under treatment with GPB and followed-up by our center.

Patient n	Diagnosis	Genetic analysis	Switched from	Age at GPB start	Dose GPB	Associated drugs	Notes
7	OCT	Gene OTC: het c.674C>T	Na benzoate	10.5 years	3 ml × 3 or 280 mg/kg or 7.9 ml/mq	Arginine	Plasma Glutamine 842-1019 kg before switch to GPB and 685-851 after switch
8	OCT	Gene OTC: het c.422G>A	NaPB	21.5 years	5 ml × 3 or 8.6 ml/mq	Citrulline, Na benzoate	Low compliance Plasma glutammine: 1,019 µmol at switch to GPB and 820 µmo/L after 1 year
9	Citrullinemia	Gene ASS: het c.814C>T/ IVS5+1G>A	NaPB	11 years	2 ml × 3 or 205 mg/kg or 5.3 ml/mq	Arginine	Plasma glutammine: 623-1032 µmol before and 576-1057 after switch to GPB
10	OCT	Gene OTC: het c.386G>A	Na benzoate	17 years	3.5 ml × 4 or 220 mg/kg or 7.3 ml/mq	Citrulline, arginine	Before switch: PT 57-92% INR 1.15-1.45 After switch: PT 77-92 INR 1.05-1.19
11	Argininosuccinic aciduria	Gene ASL: homo c.857A>G	Na benzoate	17.5 years	3.5 ml × 4 or 220 mg/kg or 7.3 ml/mq	Arginine	Plasma glutammine: 689-474 µmol/l before switch to GPB 510-793 µmol/ l after switch to GPB
12	OCT	Gene OTC: het c.928G>T	NaPB	35 years	3.2 ml × 3 or 207 mg/kg or 6.4 ml/mq	Citrulline, Na benzoate	Urine orotic acid: Before 405 mmol/ mol creatinine After- 24.14 mmol/mol creatinine

ing, hallucinations, paranoia, and mania, while chronic hyperammonemia manifests with growth failure, confusion, lethargy, dizziness, vomiting, hyperactivity, mood alteration, behavioral changes, and aggressiveness[3].

The long-term UCD management goal is to prevent hyperammonemia and achieve normal physical and mental development. Treatment consists of a combination of a low-protein diet, citrulline or arginine supplementation, and nitrogen-scavenging drugs, such as sodium benzoate, sodium phenylbutyrate (NaPB), and glycerol phenylbutyrate (GPB)[3]. NaPB is the most frequently used drug, but the regimen is challenging because of the poor palatability and volume and frequency of administration[3].

GPB is currently approved for use in the USA and Europe for patients of all ages with UCD who cannot be managed with protein restriction and/or amino acid supplementation alone. GPB consists of a tasteless, odorless, sugar and sodium-free liquid and is administered in a small volume, resulting in increased ease of the therapeutic regimen[5].

The efficacy of GPB was initially demonstrated in a pivotal phase III, randomized, double-blind, crossover trial in adult UCD patients. GPB was not inferior to NaPB compared to ammonia control (the mean 24-hour area under the curve was 866±661 *vs*. 977±865 μmol× h/L, respectively)[6]. In addition, GPB was proven to be safe and effective in early UCDs for children's short- and long-term treatment. In patients from 2 months to 17 years of age, as well as in a case series between 2 months and 2 years of age, the efficacy of GPB on 24-hour ammonia exposure was found to be non-inferior to that obtained with NaPB, while long-term treatment with GPB was associated with normal Glutamine and essential amino acids levels, age-appropriate growth and fewer hyperammonemic crises compared with the 12-month period preceding enrollment[7,8]. Finally, control of ammonia and Glutamine was maintained, and the treatment was tolerated in infants <2 months of age, switched from NaPB to GPB[9].

In the short- and long-term treatment, the most frequent adverse events observed in clinical studies and related to therapy with GPB were neutropenia, vomiting, diarrhea, pyrexia, hypophagia, cough, nasal congestion, rhinorrhea, and rash. Most treatment-related adverse events (TEAs) reported were mild to moderate in severity[6-8].

Based on the evidence from clinical studies, GPB is an effective and tolerated nitrogen-scavenging drug with characteristics enhancing treatment adherence and improving patients' and caregivers' quality of life. Nevertheless, due to the rarity of UCDs, the literature discussing the best use of GPB in clinical practice is scant, and clinicians may require insight into this field. In this setting, sharing experience in the diagnosis and management of patients may provide information that can complement high-level evidence.

Case Presentation

Case 1

Case description

- OTC deficiency: male
- Prenatal diagnosis was rejected for religious reasons.
- Confirmed by genetic analysis (hemizygote pathogenetic variant c.717g>A in the OTC gene), some days after birth.
- Family history: Maroccan origin (ancestry). The mother heterozygote for the pathogenetic variant c.717 g>A in the OTC gene; a male cousin with neonatal onset OCT deficiency; a female cousin with OTC deficiency; a mother's sister manifesting carrier of hyperammonemia in adolescence; a mother's sister with mitochondrial encephalopathy (BCS1 gene) and severe neurologic impairment.
- Preventive treatment at birth: stopped protein intake, glucose infusion, sodium benzoate, citrulline, followed by re-feeding and GPB at normalization of orotic acid.
- No newborn hyperammonemia.
- Six hospitalizations and 4 metabolic failure events due to infections in the first year (emergency protocol for management of acute hyperammonemia as in guidelines[3])
- Persistent good metabolism.
- At 13 months, the cognitive development was consistent with the age.

A child was born in a family with a history of OTC deficiency. The mother was affected with a classical type deficiency of OTC (heterozygote for the pathogenetic variant c.717 g>A in the *OTC* gene). She was on a low-protein diet and pharmacological substitutive therapy with citrulline. The same OTC deficit was present in two of the mother's sisters.

A 9-year-old male cousin had shown an early onset OTC deficiency with a neonatal hyperam-

monemic coma and liver failure. The acute event was treated pharmacologically, and the child underwent oral detoxifying therapy with sodium benzoate and NaPB, substitution therapy with L-arginine and citrulline, and a strict low-protein diet. He received a liver transplant at the age of 3 years.

A 6-year-old female cousin had a deficiency of OTC and was treated with citrulline while on an unrestricted diet. A mother's sister was affected by mitochondrial disease with severe neuromotor and cognitive deficiency, spastic quadriplegia, sensorineural hearing loss, and secondary epilepsy for a deficiency of complex III of the mitochondrial enzymatic respiratory chain due to homozygote mutation c.550C>T on the *BCS1* gene.

Notwithstanding the anamnesis, prenatal diagnosis was not performed, but the mother's plasma amino acid profile was monitored during pregnancy. Citrulline level progressively decreased to the lower value of 6 µmol/L (normal value 17-53): this finding suggested that the child could consume maternal citrulline and be affected by OTC deficiency.

At birth, blood ammonia was 91 µmol/L (normal value <110). The plasma amino acid profile showed normal levels of citrulline and arginine and a high level of ornithine. The urine level of orotic acid was extremely high, 845 mmol/mol creatinine (normal value <5.5). Glucose infusion of 8 mg/kg/minute by venous umbilical catheter was immediately initiated. Since OTC deficiency was suspected, protein intake was discontinued, and therapy with 250 mg/kg/day sodium benzoate and 200 mg/kg/day oral citrulline was started. The orotic acid level in urine was normalized in 24 hours. The therapy was modified based on the serial assessment of the plasma amino acid profile. Sodium benzoate was discontinued after the occurrence of hypoglycinemia, and 5 ml/m^2 GPB was initiated. The dose of citrulline was progressively adapted to the body weight up to the current dose of 250 mg/kg/day. Protein intake was reintroduced up to the age-safe amount. Glucose IV infusion was gradually discontinued.

During re-feeding, transient caloric support with 2% maltodextrin was necessary. The molecular analysis showed the hemizygote pathogenetic variant c.717g>A in the *OTC* gene and allowed the diagnosis of OCT deficiency.

The molecular analysis confirmed the diagnosis of OTC deficiency, showing the hemizygote pathogenetic variant c.717g>A in the *OTC* gene. Low protein diet and supplementation of citrulline were continued.

Auditory brainstem response was assessed at 1 month, and it was compatible with bilateral profound sensorineural hearing loss. Urine cytomegalovirus and maternal-specific IgG, as well as the presence of cytomegalovirus genome in the newborn blood, were negative. The molecular-genetic study of the *BCS1* gene showed the heterozygote mutation c.550C>T, so the child was found to be a carrier. A brain magnetic resonance, studying the auditory pathways, was normal.

The child was hospitalized six times in the first year due to vomit. During four of these events, he had acute metabolic failure with hyperammonemia (336, 139, 225, and 190 µmol/L, respectively). He received IV detoxifying treatment with sodium benzoate and arginine hydrochloride (starting dose: 250 mg/kg in 2 hours; maintenance dose: 250 mg/kg in 24 hours), oral GPB (starting dose: 250 mg/kg; maintenance dose: 250 mg/kg/day in four administrations), and IV 7 mg/kg/minute glucose infusion. Protein intake was discontinued for 24 hours and progressively reintroduced. Ammonia levels were rapidly restored in all episodes, and the child showed no liver failure or increased liver enzymes. One of these episodes was linked to the infection with SARS-CoV-2, without respiratory disease or systemic impairment.

During the long-term follow-up, glutamine, glycine, and alanine levels were always stable; the arginine level was at the lower level of the normal range, and citrulline levels were low as expected; orotic acid level in the urine was out of the normal range only in two episodes of hyperammonemia (28.62 and 26.02 mmol/mol of creatinine, respectively). Protein intake and drug dosages were adapted to the body weight increase.

At the age of 13 months, the raw score of the Bayley Scales of Infant and Toddler Development, third edition, was 42, and the cognitive development was consistent with the age[10].

In conclusion, the child affected with classic OTC deficiency survived the newborn stage without hyperammonemia failure due to the pharmacologic and nutritional preventive treatment. The metabolism was always satisfactory at later controls, and no organ damage was observed. Nevertheless, the child had four metabolic failure events linked to infections and had to be hospitalized and receive pharmacologic lifesaving treatment.

Case 2

Case description

- Type I citrullinemia: male.
- Prenatal diagnosis.
- Genetic analysis: heterozygous variants c.814C>T and IVS5+1G>A on the gene arginine-succinic synthetase.
- Family history: a twin brother was not affected, an older sister had citrullinemia.
- Preventive treatment at birth: (stop protein intake), sodium benzoate and bioarginine (continued at home with low protein intake).
- No metabolic decompensation with hyperammonemia in newborn and in the first years.
- For 2 years, poor therapeutic and dietary compliance lead to frequent hospitalization, and nutrition through nasogastric tube.
- From 3 to 4 years, 3 hospitalizations for metabolic failure (one with septic shock), prolonged day-hospital care, poor metabolic balance, nasogastric use was continued.
- At 5 years, switch from NaPB to GPB.
- Improvement of metabolic balance and nutrition, no more hospitalizations.
- At 7 years, the psychomotor and cognitive development is normal.

Due to the presence of an older sister affected with citrullinemia, a prenatal diagnosis of type I citrullinemia with the composite heterozygous variants c.814C>T and IVS5+1G>A on the gene arginine-succinic synthetase (ASS) was made on a fetus during a twin pregnancy. The twin fetus was not affected. Soon after at-term delivery, treatment was initiated in both newborns with protein suspension in the diet and oral administration of sodium benzoate and bioarginine. Blood samples obtained at birth and at 6 hours of life showed a high citrulline level in the affected child (240 and 508 μmol/l, respectively) and normal levels in the brother (38 μmol/l). The non-affected child was restored to a free diet within 12 hours from birth and was discharged without pharmacological therapy. The affected child was discharged some days later without neonatal metabolic decompensation during hospitalization. He was prescribed a low-protein diet, bioarginine and sodium benzoate oral treatment and was followed-up with regular clinical and biochemical assessments.

At 1 year, as plasma levels of Glutamine and urine levels of orotic acid were repeatedly found over the normal range (orotic acid maximum value 421 mmol/mol creatinine; n.v. 0.7-5.5), treatment with NaPB started but only partial improvement of metabolic balance was obtained.

During the following 2 years the child was hospitalized several times for clinical and poor metabolic compensation due to poor therapeutic and dietary compliance. During this period, for almost 2 years, the child was fed enterally by nasogastric tube both in the hospital and at home, maintaining a good health.

During the third year of life, the child was admitted to the hospital three-times for hyperammonemic metabolic decompensation and received intravenous detoxifying treatment. One of these episodes resulted in septic shock, disseminated intravascular coagulation (DIC) and cerebral coma, requiring referral to the pediatric intensive care unit.

The following year, although ordinary hospitalization was unnecessary, the child had several months of day-hospital care. The metabolic balance was poor, with frequent episodes of massive orotic aciduria (maximal value 421 mmol/mol creatinine; normal value 0.7-5.5), although blood ammonium and serum Glutamine levels were always within normal limits. Furthermore, enteral diet supplementation by nasogastric tube was necessary due to the suboptimal metabolic condition and reduced growth.

At the age of 3 years and 6 months, NaPB was stopped and GPB was introduced, continuing sodium benzoate and L-arginine. The switch took place overnight with the transformation coefficient: g NaPB × 0.81 = g GPB.

After the initiation of GPB the metabolic balance and the nutrition condition were improved; no further hospitalization was necessary, and some infective events were treated at home without hyperammonemic metabolic decompensation. Levels of urine orotic acid reached normal values, and plasma Glutamine remained in the normal range.

The child also resumed feeding regularly and the nasogastric tube stopped feeding; the body weight growth curve stabilized at the 10th percentile. The child is at present 7 years and 10 months old, and his psychomotor and cognitive development is normal.

Case 3

Case description

- OTC deficiency: female.
- Diagnosis at 3 years.
- Genetic analysis: pathogenic variant c.422G>A in the state of hemizygosity.

- Family history: negative.
- Clinical presentation at 3 years: recurrent vomiting, hyperammonemia, increased level of orotic acid in the urine.
- Treatment: protein intake control, sodium benzoate, citrulline, and later addition of NaP.
- For some years, poor metabolic balance, low dietary compliance, scholastic difficulties, and cognitive delay.
- Several episodes of hyperammonemia up to 10 years.
- At 14 years, switch from NaPB to GPB, continuing sodium benzoate and citrulline.
- Improvement of metabolic balance, spontaneous nutrition, cognition and psychomotor development.
- At 18 years, the study curriculum is normal.

A girl was born from an asymptomatic OCT carrier mother and presented a normal psychomotor development with the physiological acquisition of developmental milestones; she was fed both her mother's milk and formula milk. Since weaning, she has always been on a free diet. She always refused to eat meat.

At the age of 3 years and 6 months, she was hospitalized for recurrent vomiting and, following the finding of hyperammonemia, with a plasma amino acid profile characterized by hypo-citrullinemia and increased values of orotic acid in the urine, the diagnosis of OTC deficiency was made, which was then confirmed by molecular analysis of the *OTC* gene which showed the presence of the pathogenic variant c.422G>A in the state of hemizygosity. Therefore, a protein-deficient diet with amino acid mixtures and oral detoxification therapy with sodium benzoate and citrulline were introduced.

During the first years after the diagnosis, with long-term treatment, the metabolic balance was poor, dietary and drug compliance was very low, and scholastic difficulties were reported during primary school. The therapy was progressively optimized, and NaPB was added due to the frequent findings of greatly increased plasma Glutamine values.

At the age of 9 years, a neuropsychiatric evaluation demonstrated slight cognitive delay and logopaedic and psychomotor treatment with school support were undertaken.

Up to the age of 10 years, the girl presented numerous episodes of hyperammonaemia decompensation, requiring hospitalization and IV detoxification treatment.

From the age of 10 years, no further episodes of hyperammonemia occurred, but the metabolism was always not optimal due to increased values of Glutamine in the plasma.

At the age of 14 years, NaPB was replaced by GPB, while sodium benzoate and citrulline therapy were unchanged. The switch took place overnight using the transformation coefficient: g NaPB × 0.81 = g GPB.

Since the introduction of GPB, metabolic and nutritional control has improved; the girl has begun to eat more regularly, and synthetic amino acids have been suspended. The psychomotor and cognitive development has improved, and academic performance has normalized, allowing for a normal curriculum (scientific high school and university enrolment at 18 years).

Case 4

Case description

- Argininosuccinic aciduria. Male.
- Diagnosed at 4 days of life for metabolic decompensation with severe hyperammonemia.
- Genetic analysis: heterozygote pathogenetic variants c.649C>T/c.638G>A in the ASL gene.
- Family history: negative.
- Presentation: at 4 days, seizures, hyperammonemia, brain damage at imaging assessment.
- Initial treatment: sodium benzoate, chloride arginine, stopped protein intake, peritoneal dialysis.
- Long-term treatment: carnitine, NaPB, L-arginine, antiepileptic therapy, low-protein diet, and synthetic amino acid supplementation.
- At 4 months, switch from NaPB to sodium benzoate (due to vomiting).
- Mild liver failure.
- Ten hospitalizations up to 13 years due to metabolic failure and liver failure with hepatomegaly.
- Metabolic control was consistently poor, and serious mental retardation was demonstrated in these years.
- At 13 years, switch from sodium benzoate to GPB.
- Improvement of liver function markers and of clotting factors.
- At 15 years, the improvement is maintained.

This male child, after an asymptomatic interval and from the second day of life, showed feeding problems and progressive lethargy; on his fourth day of life he showed seizures and hyperammonemia with metabolic decompensation was

demonstrated. Detoxifying therapy with sodium benzoate and chloride arginine was started. Protein intake was discontinued, and peritoneal dialysis was performed. Brain magnetic resonance showed relevant injury to the putamen, pallidus, head of the caudate bilaterally, cerebellar peduncles, subcortical white matter, and both semiovale centers. A later neuroradiological control showed damage evolution, with hyperintensity areas located at the peninsular cortico-subcortical junction, the semiovale centers, and the posterior white matter of pallidus nuclei bilaterally. Repeated electroencephalograms (EECs) demonstrated a disorganized pattern with paroxysmal multifocal activity.

Long-term treatment was introduced with carnitine, NaPB, L-arginine, antiepileptic therapy, and a low-protein diet (with a total intake of 1.5 g/kg/day of natural proteins and synthetic amino acids).

Argininosuccinic aciduria was diagnosed and confirmed by genetic examination, demonstrating double heterozygote pathogenetic variants c.649C>T/c.638G>A in the argininosuccinate lyase (ASL) gene.

At 4 months, NaPB was discontinued, and sodium benzoate was introduced due to several episodes of vomiting concurrently with therapy administration and associated with hyperammonemic metabolic decompensation. Compliance with therapy was improved.

Since the disorder's manifestation, transaminase levels have been elevated with mild liver failure (the highest value of SGPT was 1623 IU/L) and hypokalaemia (treated with supplementation therapy with aspartate potassium). Hepatomegaly and liver steatosis were observed with several ultrasound scans of the abdomen.

The child was hospitalized 10-times from birth to 13 years of age due to hyperammonemic metabolic decompensation, acute liver failure, liver cytolysis with increased transaminases and hypokalaemia. All these episodes were treated with detoxifying IV therapy, increased intake of potassium aspartate, and changes in the schedule of the antiepileptic drug. At follow-up in the outpatient clinic, chronic hepatopathy was consistently present and confirmed by an ultrasound scan showing increased liver size, increased parenchyma echogenicity, and hyperechoic areas resulting in fatty liver areas.

Plasma Glutamine and glycine levels were frequently high in the blood, suggesting an unsatisfactory metabolic control, although orotic acid levels were normal.

When he was 13 years old, sodium benzoate was substituted with GPB 7.2 ml/m^2, corresponding to 216 mg/kg, in four administrations/day, using the formula conversion: GPB (mL/m^2/day) = 0.0155 NaBz (mg/kg/day) + 3.7809[11]; transaminases reached normal levels before discharge. Glutamine was normal after one month, glycine was still elevated after 10 months, but reached normal level after 16 months, and citrulline's high level persisted at later controls. Arginine and coagulation factors were within normal values before the switch and remained so in the following controls. The use of milk supplemented with medium-chain triglycerides was discontinued, improving dietary compliance without changes in blood chemistry values.

In summary, the patient had severe hyperammonemic decompensation with brain damage as a newborn and developed chronic liver disease, serious mental retardation with an absence of speech, and difficult nutrition over time. In addition, he underwent several episodes of acute hyperammonemia and acute liver failure with high transaminase levels. After the introduction of GPB, transaminase and clotting factor levels were repeatedly found to be normal; as GPB has only been used for 2 years at the time of this report, this observation needs to be further confirmed over a longer period of time.

Case 5

Case description

- OTC deficiency. Female.
- Diagnosed at neonatal age.
- Genetic analysis: not available as it was performed in another laboratory
- Family history: older brother dead of acute neonatal hyperammonemic decompensation.
- Normal diet and neurodevelopment.
- At 3 years: transaminase values persistently at the upper limits with alterations in liver function and increased levels of urinary orotic acid.
- Treatment: sodium benzoate without metabolic improvement, and addition of NaPB, protein restriction, synthetic aminoacids, and vitamin K.
- At 5 years and 3 months, switch from NaPB to GPB.
- Improvement of hepatic markers and coagulation and normalization of Glutamine and orotic acid levels; discharged aminoacid formula
- Day-hospital attended over 10 times/year before GPB, and 2 times/year after GPB introduction
- Follow-up to 9 years old.

The diagnosis of OTC deficiency was made in a girl in the neonatal period, following investigation due to family history with familiarity with her mother (the genetic analysis was performed in another center and was not available), an older brother who died of acute neonatal hyperammonemic decompensation and an older sister affected. At birth, the patient was fed the mother's milk, normal protein intake, and started pharmacological treatment with bioarginina. The child had normal psychomotor development, with adequate neuromotor milestones and language acquisition.

At 3 years of age, transaminase values were persistently at the upper limits with alterations in liver function and increased levels of urinary orotic acid; therefore, protein restriction in the diet was prescribed. As increased transaminase blood levels and coagulopathy persisted, therapy with oral sodium benzoate was introduced without improvement of signs of chronic liver disease: after further worsening of the condition, with high values of urinary orotic acid (650 mmol/mol cr) and plasma Glutamine (about 1200 µmol/l) sodium benzoate therapy was associated with NaPB and protein restriction was implemented.

Due to persisting poor metabolic control, high plasma Glutamine (always between 900 and 1260 mmol/l), and the stable alteration of coagulation parameters, low natural protein intake and diet supplementation with daily oral synthetic amino acids and vitamin K were introduced. Biochemical parameters further worsened notwithstanding this treatment, and NaPB was replaced with GPB, at the age of 5 years and 3 months. The switch took place overnight using the transformation coefficient: g NaPB × 0.81 = g GPB.

Since the introduction of GPB in February 2019, hepatic parameters have markedly improved, and the hepatic cytolysis indices were normalized. The coagulation was completely restored, which made it possible to stop therapy with vitamin K. After the switch to GPB, the girl no longer presented coagulopathy, with prothrombin activity and INR (International Normalized Ratio) values stably in the range.

The dosage of GPB was progressively optimized until obtaining persistent normalization of plasma Glutamine and urinary orotic acid values.

Considering the improved metabolic compensation, the intake of synthetic amino acid mixtures was gradually reduced until complete suspension, and intake of all natural proteins was reintroduced.

While 21 days of day-hospital admissions due to poor metabolic balance had been necessary for the 2 years before GPB initiation, only follow-up every 6 months has been performed up to the age of 9 years.

Case 6

Case description

- OTC deficiency. Female.
- Diagnosis at 15 months.
- Genetic analysis: hemizygote *de novo* mutation c.626 C>T p.(Ala209Val) of the *OTC* gene.
- Family history: negative.
- Normal but slow development in the first year.
- Vomit episodes and refusal of meat after 1 year.
- At 15 months, acute serious vomiting, hyperammonemia, high transaminases, low plasma citrulline, and high urine orotic acid.
- Treatment: arginine chloride and sodium benzoate, stopped protein intake; dose adjustment and addition of NaPB due to poor metabolic balance. NaPB was not easily accepted due to the bad taste of granules and powder.
- No improvement, and 30 daily hospitalizations in the first 20 months of treatment.
- At 2 years and 8 months, switch from NaPB to GPB, with citrulline and arginine. Followed by discontinuation of sodium benzoate and L-arginine after 1 year.
- Normal liver function; decreased frequency of hospitalizations.
- The psychomotor development was always normal.
- At 6 years and 4 months, the girl regularly attends school.

A female child was born from a twin dichorionic and bi-amniotic pregnancy. The growth and motor development were regular in the first year, but growth was slow after this age. In the second year, she had repeated vomit episodes, not linked to infection, associated with reduced reactivity and refusal of meat.

At 15 months, an acute event with uncontrollable vomiting and hypo-reactivity occurred. Laboratory tests demonstrated 289 µmol/L blood ammonia (normal level <50), 5730 UI/L aspartate aminotransferase (normal value 8-60), 4811 UI/L alanine aminotransferase (normal value 7-45), 21% prothrombin time (normal value 70-100), International Normalized Ratio 3.2 (normal value <1.25), showing liver cytolysis and acute liver failure. The plasma citrulline level was 1 µmol/L

(normal value 17-53), and the arginine level was 25 μmol/L (normal value 38-135). The urine orotic acid level was 17.33 mmol/mole of creatinine (normal value 0.7-5.5). A gene analysis was performed as these findings suggested a possible OTC deficiency. A hemizygote *de novo* mutation c.626 C>T p.(Ala209Val) of the *OTC* gene was found.

According to international guidelines, IV detoxifying therapy with arginine chloride and sodium benzoate was started, and a protein-free diet was introduced[3]. Ammonium and coagulation factors were normalized. Long-term pharmacologic treatment with L-arginine and sodium benzoate was initiated, with a diet with 1.1 g/kg/day protein intake.

Plasma Glutamine and urine orotic acid levels were consistently increased during the follow-up, and drug dosages were changed to restore metabolic control. Additionally, the protein intake was reduced to the safety level for the age. Citrulline 200 mg/kg was added, followed by the addition of 250 mg/kg/day NaPB, without beneficial changes in laboratory test values. The child had great difficulty in taking NaPB, either powder or granules, the management of diet and pharmacological treatment was very difficult for the family, social life was restricted and the child could not attend nursery school.

The child was hospitalized about 30 times during the first 20 months of follow-up, greatly impacting her quality of life.

At 2 years and 8 months, she started GPB (starting dose 4.6 ml/m^2 and maintenance dose 6.9 ml/m^2), with citrulline and arginine, and discontinued NaPB. The compliance was excellent. L-arginine and sodium benzoate were discontinued in 1 year. Liver function was always normal. Due to acute infections, the child had only two metabolic failure episodes, requiring hospital admission between November 2019 and September 2021.

The cognitive development was always within the normal range. At 2 years and 7 months, the Bayley Scales of Infant and Toddler Development composite scores, third edition, were 95 in the cognition area, 83 in the language area, and 85 in the motor area.

The Wechsler Preschool and Primary Scale of Intelligence, third edition, was administered at the age of six years. The performance intelligence quotient was 111 (percentage rank 77; 95% CI: 102-118), and the processing speediness quotient was 112 (percentage rank 79; 95% CI: 102-119); the verbal area items could not be administered.

The number and frequency of hospital admissions have decreased after the initiation of GPB; the child entered the school at regular age and is currently (2 years after switch to GPB) attending school regularly. She is practicing sporting activities.

Discussion

This article presents 6 hexemplary cases of patients with UCD, who were successfully treated with GPB. These patients had different types of UCDs, including OTC deficiency, citrullinemia, and argininosuccinic aciduria, were diagnosed in different life phases (different ages), and GPB was initiated at different ages (newborn – 14 years). Additionally, the family history was positive in 3 cases and negative in the other ones.

GPB was always introduced after previous treatments, which had proven unable to ensure optimal control of the disease. Overall, treatment with GPB was followed by a relevant metabolic improvementresulting in: a) better therapeutic compliance in all patients exept one (patient No. 1 who was a newborn); b) reduced number of hospitalization (especially in patients 2, 5 and 6); c) improved quality of life, generally in all patients but especially in number 2 (discharged nasogastral feeding and normalized alimentation by mouth), in patients number 5 and 6 (dramatic reduction of number of hospitalization). Excepting the patient number 4, who had a relevant neurologic damage since the manifestation of the disease occurred at neonatal age, all patients achieved a satisfactory or normal cognitive development and were able to attend school, university and normal life activities like sport or social life.

A strict monitoring of patients allowed dose and regimen adjustments along the life of children, preventing serious impairments of functionality. Restoration of a satisfactory metabolic balance resulted in prevention of organ damage and allowed a good development and growth. Normalization of transaminases and of liver function was achieved and maintained in the long-term.

Thus, as effective treatment is availble for the prevention of organ damage, it is important that physicians may facilitate early and timely diagnosis, and referral to specialized centers.

The efficacy and safety of GPB have been proven by clinical trials, and experiences in the clinical practice have shown benefits in terms

of wellbeing and long-term quality of life[6-9,12]. The aim of long-term treatment is to ensure a stable metabolic control, with normal levels of ammonia and Glutamine. To achieve this goal it is not enough to have an effective therapy; indeed, patients must be compliant with such therapy.

As previously reported, GPB is welcomed by patients because of its low volume, palatability, and easy administration[13], while reluctance to take NaPBA may be due to poor taste and large volume[14]. Acceptance of the medication contributes to adherence, allowing the full efficacy of management. GPB is usually well-tolerated, also contributing to adherence and good outcomes[13]. A Spanish study[12] in the real life setting demonstrated that after switching to GPB from another nitrogen scavenger, ammonia and Glutamine levels and the frequency of hyperammonemia were significantly reduced.

A qualitative study found that adhering to a strict dietary and medication regimen is extremely stressful for both caregivers and children with UCDs, with both parties having to miss out many events in their social lives, with severe impact on the psychological well-being of the family[14]. The authors found also that switching to GPB was perceived to have alleviated the burden of treatment in terms of better characteristics concerning taste and volume.

Among our cases, nutrition was improved after the switch to GPB, thanks to low frequency of vomiting and improved wellbeing; in one case, nutrition through nasogastric tube could be stopped. All this allowed discontinuation of supplements such as medium-chain triglycerides or synthetic amino acid, and improved the quality of life of children and their caregivers. During long-term follow-up, dose escalation of GPB, when required, is simple because the medication is almost tasteless and odourless, contains no sugar or sodium, and is administered in relatively low volumes[13].

GPB is well tolerated by newborns, without risk of hypernatiemia. Early administration in newborns prevented metabolic decompensation, and allowed early normal oral feeding, without long hospitalizations.

Conclusions

In agreement with the literature, we describe some cases of children with UCD, who had improved metabolic control, which in turn allowed these patients to achieve a better quality of life after switching to GPB, facilitating normal life activities, development and growth.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Authors' Contribution

Data recovery: GS, AD. Drafting and approval of manuscript: GS, AD.

Ethics Approval

The content of this article has been notified to the local ethics committee on September 28th 2023.

Informed Consent

Informed consent was obtained from parents of all individual participants included in the study.

Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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