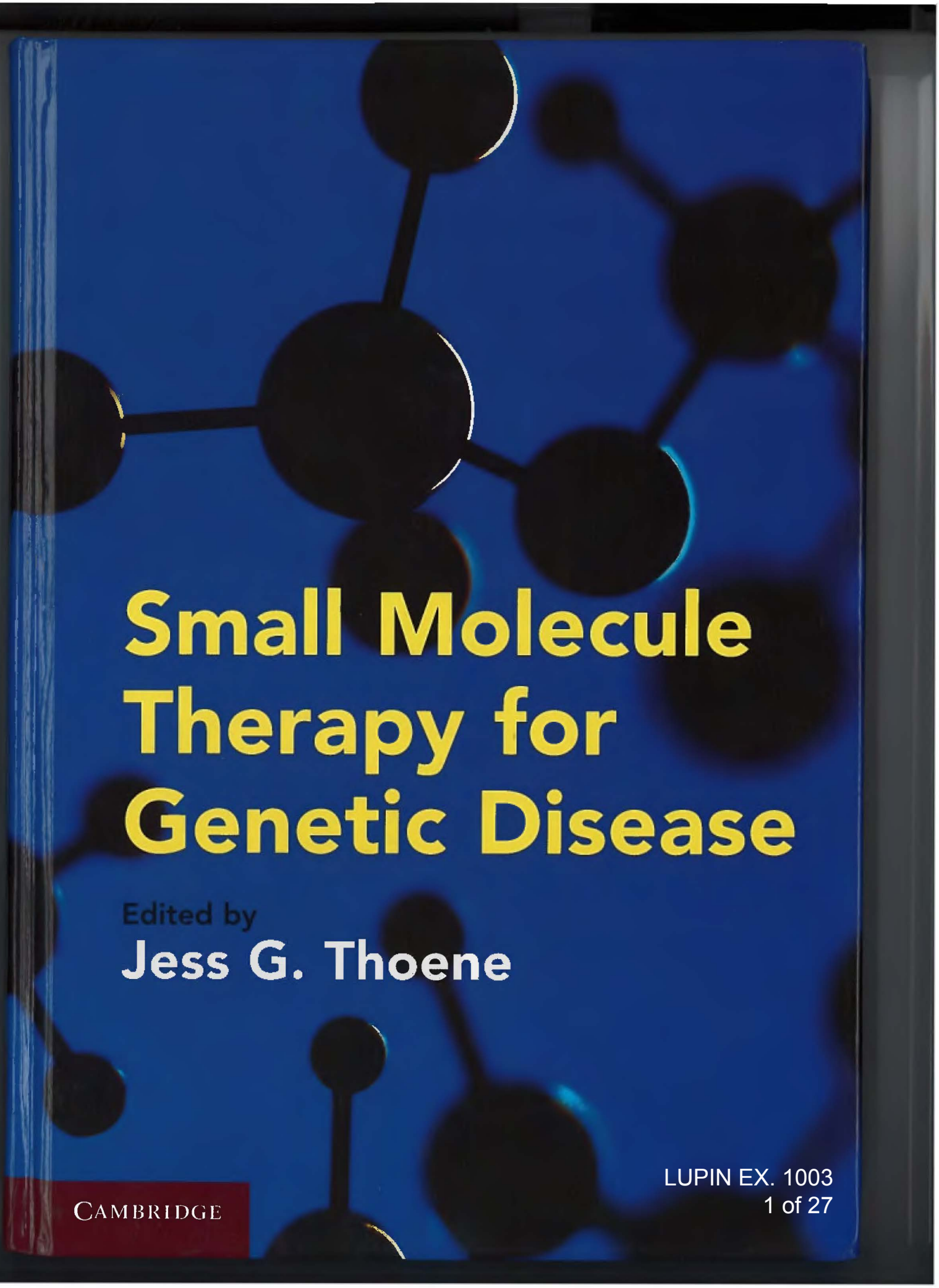

SMALL MOLECULE THERAPY FOR GENETIC DISEASE

This book summarizes the substantial work that has been accomplished with simple molecules in the treatment of inborn errors of metabolism. These agents are discrete, often of natural origin, and provide predictable therapeutic responses. As such, they avoid many of the practical difficulties associated with gene and protein therapies.

This book will enable interested clinician/scientists and others to rapidly survey the field, thus ascertaining what has been done as well as future directions for therapeutic research. Its important introductory chapters discuss the infrastructure of the field. These chapters focus on an introduction to pharmacokinetics and pharmacodynamics, a description of the FDA Office of Orphan Products, and a summary of the operation of the National Institutes of Health Office of Rare Diseases Research. The remainder of the book is devoted to a review of small molecule therapy for genetic diseases. The book closely analyzes the cofactors used to augment the function of defective enzymes and the compounds that are able to use an alternative pathway to avoid the consequences of the metabolic block present in the patient. Among other therapies, the authors discuss the use of zinc and tetrathiomolybdate to treat Wilson disease and the use of cysteamine to treat nephropathic cystinosis.

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Small Molecule Therapy for Genetic Disease

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10 Alternative waste nitrogen disposal agents for urea cycle disorders

Gregory M. Enns

NATURAL HISTORY OF UREA CYCLE DISORDERS

Urea cycle disorders (UCDs) are inborn errors of metabolism characterized by episodic, life-threatening hyperammonemia secondary to partial or complete inactivity of enzymes responsible for eliminating nitrogenous waste. The urea cycle was initially elucidated by Krebs and Henseleit in 1932.[1] In 1958, argininosuccinic acid lyase deficiency became the first enzymatic defect of the urea cycle to be identified, and reports of all others, except *N*-acetylglutamate synthetase (NAGS) deficiency, followed in the 1960s.[2] Ornithine transcarbamylase (OTC) deficiency is the most common UCD, followed by argininosuccinate synthetase (AS) deficiency (citrullinemia), carbamoyl phosphate synthetase (CPS) deficiency, and argininosuccinate lyase (AL) deficiency. NAGS deficiency was first described in 1981 and has been documented in only a few patients.[3] Estimates of overall incidence of UCDs in the United States have ranged from approximately 1 in 25,000 to 1 in 8,200 births.[4,5]

Historically, mortality and morbidity have been high, with survivors commonly showing devastating neurological sequelae.[2] UCDs are the most common cause of neonatal hyperammonemia and typically present with symptoms of poor feeding, lethargy, hypotonia, irritability, seizures, respiratory distress, grunting, and hyperventilation. Other disorders common in neonates, such as sepsis, cardiac failure, and intracranial hemorrhage, are also in the differential diagnosis because similar clinical findings may occur in these conditions. Therefore, in all neonates presenting with nonspecific symptoms of distress, a plasma ammonium level should be obtained. If the level of ammonium is elevated, diagnostic evaluations and treatment should be started immediately.

Although presentation in the neonatal period has been well-documented, patients who have a partial enzyme deficiency typically manifest after the neonatal period. UCDs may strike at any age. Indeed, approximately two thirds of cases initially present after the neonatal period.[5] Clinical features may be subtle in such late-onset cases, leading to delays in diagnosis. In addition to acutely altered mental status, later-onset patients may have episodic ataxia, psychiatric and behavioral symptoms, psychomotor delay, and gastrointestinal complaints, such as loss of appetite and episodic emesis.[6]

Many survivors of the initial hyperammonemic episode undergo recurrent attacks of hyperammonemia requiring hospitalization. Such episodes are typically preceded by an illness, especially a viral syndrome. Other events that contribute to hyperammonemic episodes include dietary or medication noncompliance and major life events, such as surgery, gastrointestinal bleeding, accidents, school stress, or parturition. Catabolic stress from viral illnesses appears to be a more significant risk factor than is increased intake of dietary nitrogen for causing hyperammonemia.[5,7]

A study of 260 UCD patients showed that onset of symptoms in the neonatal period results in the worst outcome (35% survival approximately 11 years after the start of the study period), and patients who presented initially in late infancy have the best outcome (87% survival; Figure 10–1). Percent survival to the final follow-up time point was highest for patients with AS deficiency (78%), followed by girls with OTC deficiency (74%), and CPS-I deficiency (61%).[5] Boys with OTC deficiency have the lowest survival rate over time (53%), as well as the lowest survival rate following hyperammonemic crises (71%).[5,8]

Although alternative pathway therapy and other therapies, especially hemodialysis, for UCDs has led to improved patient survival, cognitive impairment remains a common finding, especially in patients who have neonatal-onset disease.[9]

PATHOPHYSIOLOGY

Ammonia is present in all body fluids and exists mainly as ammonium ion at physiologic pH. Hyperammonemia is defined as a blood ammonia concentration greater than approximately 100 μmol/L in neonates or 50 μmol/L in children

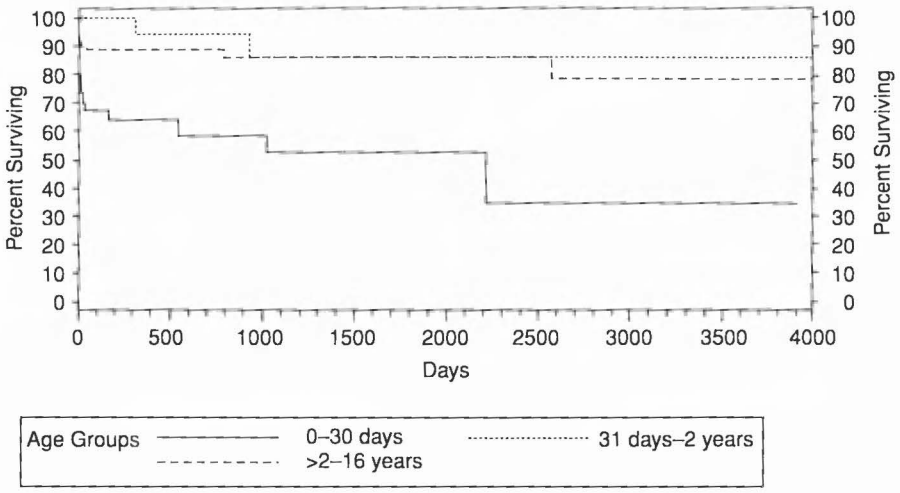

Figure 10–1: Kaplan–Meier survival by age at first episode of hyperammonemia. Survival time was calculated as the amount of time between the discharge date of the last episode and the admission date from the first episode. Only 35% of patients who presented with the first hyperammonemic episode during the neonatal period were still alive at the final follow-up time point (approximately 11 years after the start of the study period).[5] Reproduced with permission from *Acta Paediatrica*.

and adults (precise cutoffs vary depending on individual laboratory normative ranges). A five- to tenfold increase in blood ammonium levels is usually toxic to the nervous system.[10] Acute hyperammonemia causes astrocyte swelling and global cerebral edema, which affects brain white matter selectively. Changes involving the deep insular and perirolandic sulci may be reversible.[11] In cases of severe hyperammonemia, however, permanent changes occur. Neuropathological findings in patients who have neonatal-onset proximal UCDs consist of gross cerebral atrophy, ventriculomegaly, delayed myelination, the appearance of Alzheimer type II astrocytes, ulegyria, and spongiform degeneration of the cortex, gray–white matter junction, and deep gray nuclei, including the basal ganglia and thalamus.[11]

Hyperammonemia also causes increased cerebral cortical glutamine content, activation of astrocytic glutamine synthetase, and astrocyte swelling. Ammonia diffuses freely across the blood–brain barrier and is rapidly incorporated into glutamine via glutamine synthetase. Glutamine synthetase, a cytosolic enzyme primarily localized to the astrocyte in the brain, catalyzes the following reaction:

$$NH_4 + \text{L-Glutamate} + ATP \rightarrow \text{L-Glutamine} + ADP + P_i.$$

This reaction, therefore, represents a short-term means of buffering excess plasma ammonium. In theory, glutamine also may be an organic osmolyte that increases intracellular osmolarity. Such an increase in osmolarity would lead to increased cellular volume, as water enters the astrocyte, and subsequent cytotoxic cerebral edema.[9,12]

Although the "glutamine hypothesis" has been a leading explanation for the development of cerebral edema, other research has focused on glutamine-independent mechanisms to explain the pathogenesis of hyperammonemic encephalopathy. In particular, the role of impaired brain oxidative metabolism in causing cerebral dysfunction associated with hyperammonemia is an area of active investigation. Acute hyperammonemia causes a decrease in brain metabolic rate and high-energy phosphate concentration and increased production of toxic reactive oxygen species (ROS) by brain mitochondria.[13] Glutamine also enters mitochondria through a histidine-sensitive carrier. This process is potentiated by ammonia. Phosphate-activated glutaminase is located in the inner mitochondrial membrane and cleaves glutamine into glutamate and ammonia. Because of this localized production of ammonia, intramitochondrial ammonia levels have the potential to become high, leading to induction of mitochondrial permeability transition (MPT), increased oxidative and nitrosative stress, and astroglial dysfunction. The production of ROS and reactive nitrogen species and the induction of MPT have been hypothesized to initiate a cascade of events that includes activation of mitogen-activated protein kinases (MAPKs) and resultant failure of astrocytes to regulate their intracellular volume.[14]

In addition, ammonium ions have a multitude of effects on mammalian neurotransmitters, including systems involving cholinergic, serotonergic, and glutamatergic neurotransmission. The increased seizure predisposition in some UCD patients may be explained, in part, by increased brain concentrations of the excitatory amino acid neurotransmitters glutamate and aspartate. Increased levels of these amino acids are present in the sparse-fur (*spf*) mouse, a model of OTC deficiency.[15] Tryptophan, a precursor of serotonin, and quinolinic acid, an *N*-methyl-D-aspartate (NMDA) receptor agonist known to produce selective striatal cell loss, are similarly increased in *spf* mice and in rats following portacaval anastomosis. Ammonia also inhibits high-affinity transport of glutamate in astrocytes. This inhibition results in increased extracellular concentration of glutamate.[16] These biochemical and pathological findings suggest that NMDA-mediated excitotoxic brain injury may be occurring in UCD patients.[17] Ammonium ions also depress postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated currents.[16] AMPA receptors mediate fast synaptic transmission and are involved with learning and memory.

Chronic hyperammonemia activates the L-system carrier, which results in a loss of NMDA receptor densities and increased uptake of tryptophan into the brain.[16] Serotoninergic symptoms, such as anorexia, altered sleep patterns, and disorders of motor coordination, may be related to the increased brain turnover of serotonin observed in hyperammonemic states.[11] The adaptive changes in NMDA receptors that occur in chronic hyperammonemia result in a decrease in excitatory neurotransmission and impaired production of nitric oxide and cyclic guanosine monophosphate (cGMP). Decreased cGMP production may inhibit long-term potentiation (LTP) in the hippocampus. Because LTP is a long-lasting

Table 10–1. Molecular genetics of the UCDs

Disorder	Cellular compartment	Gene	Chromosomal location	Molecular characteristics
NAGS deficiency	Mitochondrial matrix	NAGS	17q21.31	7 exons, spans ~5 Kb, ORF* ~1.6 Kb, 534 amino acids
CPS deficiency	Mitochondrial matrix	CPS1	2q35	38 exons, spans ~201 Kb, ORF ~5.8 Kb, 1,500 amino acids
OTC deficiency	Mitochondrial matrix	OTC	Xp21.1	10 exons, spans ~94 Kb, ORF ~1.7 Kb, 354 amino acids
AS deficiency (citrullinemia)	Cytosol	ASS1	9q34.1	15 exons, spans ~57 Kb, ORF ~1.9 Kb, 412 amino acids
AL deficiency	Cytosol	ASL	7cen-q11.2	16 exons, spans ~79 Kb, ORF ~1.9 Kb, 464 amino acids
Arginase deficiency	Cytosol	ARG	6q23	8 exons, spans ~37 Kb, ORF ~1.4 Kb, 322 amino acids

* ORF: open reading frame.
Note: Molecular characteristics of the UCDs are shown. Data were obtained from the National Center for Biotechnology Information (NCBI) Web sites (www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html [Ace View]; www.ncbi.nlm.nih.gov/nuccore [Entrez Nucleotide]; www.ncbi.nlm.nig.gov/omim [OMIM]).

enhancement of synaptic transmission efficacy, considered to be the basis for some forms of learning and memory, this effect of hyperammonemia may be related to the abnormal cognitive function observed in patients who have UCDs.[18] Abnormal axonal growth, accompanied by decreased creatine and phosphocreatine levels (creatine is essential for axonal elongation) and alteration of brain cytoskeletal elements, are also observed in hyperammonemia. Glial fibrillary acidic protein (GFAP) is an important astrocytic protein involved in a multitude of cellular functions. GFAP is reduced, and microtubule-associated protein-2 (MAP-2) and neurofilament protein (NF-M) exhibit decreased phosphorylation, possibly through abnormal MAPK function caused by hyperammonemia.[19] Although the precise interrelationship between these proposed pathogenetic mechanisms is unclear, it is reasonable to suspect that these processes play at least some role in the mental impairment observed in UCD patients.

INHERITANCE

Urea cycle defects, with the exception of OTC deficiency, are inherited as autosomal recessive traits. OTC deficiency is X-linked and, therefore, typically manifests more severely in males. Approximately fifteen percent of females with OTC deficiency display symptoms, such as protein intolerance, cyclical vomiting, behavioral and neurologic abnormalities, and even hyperammonemic coma, and are termed "manifesting heterozygotes." The severity of symptoms in such females is related to random X-inactivation and allelic heterogeneity, as well as to degree of environmental stress. Further details regarding the molecular genetics of the UCDs are provided in Table 10–1.

EARLY EFFORTS AT HYPERAMMONEMIA THERAPY

A number of different therapies aimed at removing accumulated ammonia in cases of hyperammonemic encephalopathy have been attempted, including lactulose (reduces the production or absorption of the end products of bacterial nitrogen metabolism in the colon), exchange transfusion, peritoneal dialysis (PD), hemodialysis, and supplementation with nitrogen-free analogues of essential amino acids. Although children treated with α-keto amino acid analogues showed some clinical improvement, such as improved seizure control attention span and weight gain, death in infancy was still common. Exchange transfusions are ineffective in managing hyperammonemia. PD has shown variable efficacy in treating hyperammonemia but, in general, is far inferior to hemodialysis. The early use of these treatments prolonged survival in some cases, but overall efficacy has been disappointing.[20]

ALTERNATIVE PATHWAY MEDICATIONS

In 1914, Lewis demonstrated that sodium benzoate could divert urea nitrogen to hippurate (HIP) nitrogen in two normal subjects. After ingestion of sodium benzoate, blood urea nitrogen and ammonia levels fell and urine HIP excretion rose markedly, with little change in total urine nitrogen excretion.[21] Shiple and Sherwin later showed that oral administration of phenylacetate results in substitution of phenylacetylglutamine (PAGN) nitrogen for urea nitrogen in urine. Coadministration of benzoate and phenylacetate resulted in as much as 60% of urine nitrogen being excreted as HIP and PAGN.[22] Subsequently, the enzymes responsible for these reactions (acyl-coenzyme A [CoA]:glycine and acyl-CoA glutamine *N*-acyltransferases) were identified and localized to both kidney and liver in humans and primates. Synthesis of HIP (from conjugation of glycine with benzoate) and PAGN (from conjugation of glutamine with phenylacetate) requires adenosine triphosphate (ATP) and CoA. Pharmacogenetic factors partly determine the activity of enzymes responsible for formation of HIP and PAGN and, therefore, play a role in determining the individual rate of nitrogen removal.[23]

MECHANISM OF ACTION

In 1979, Brusilow and colleagues suggested that the use of endogenous biosynthetic pathways of non-urea waste nitrogen excretion could substitute for defective urea synthesis in patients who have UCDs. By promoting the synthesis of non-urea nitrogen-containing metabolites (the excretion rates of which are high or may be augmented), in theory, total body nitrogen load could be decreased despite abnormal urea cycle function.[24] The two classes of alternative pathway metabolites are (1) urea cycle intermediates (citrulline and argininosuccinate) and (2) amino acid acylation products (HIP and PAGN).

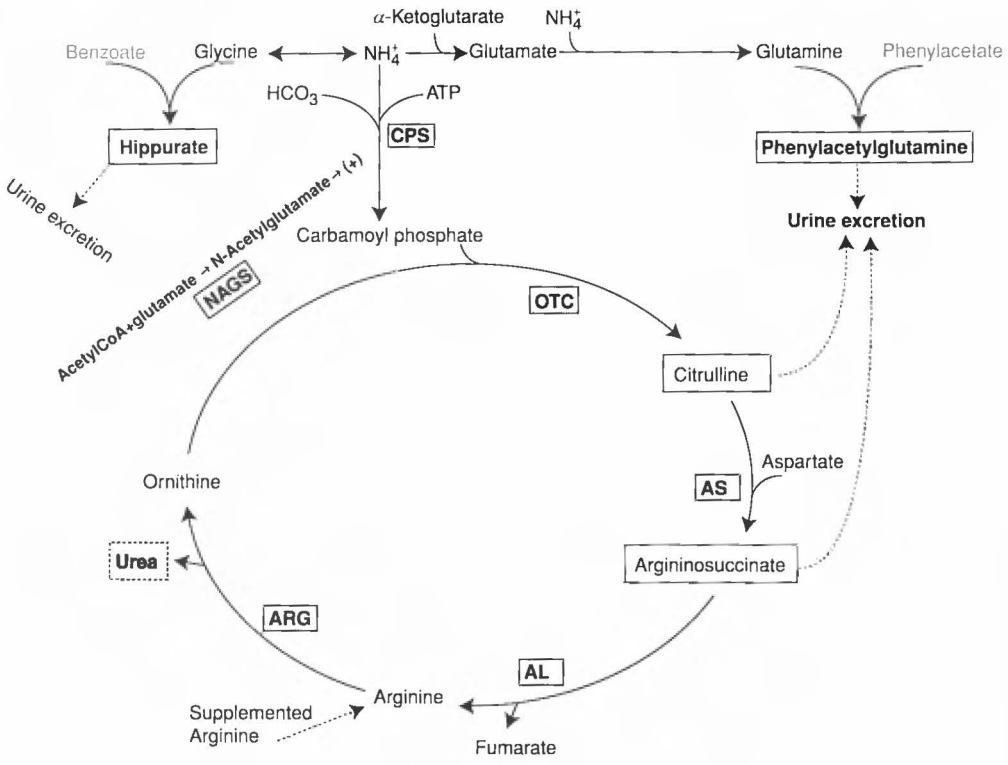

Figure 10–2: The urea cycle and alternative pathway therapy. Nitrogen flux through the urea cycle may be decreased by excretion of HIP and PAGN, molecules which contain one and two waste nitrogen atoms, respectively. In addition, citrulline (which contains one waste nitrogen atom) or argininosuccinate (which contains two waste nitrogen atoms) can serve as a vehicle of waste nitrogen excretion, depending on the location within the urea cycle of the deficient enzyme. For example, in AL deficiency, there is a block in argininosuccinate conversion to arginine. Supplementing AL deficiency patients with arginine increases production of argininosuccinate, which is then excreted in the urine along with its waste nitrogen. ARG: arginase. Reproduced with permission from *NeoReviews*, Vol. 7, page e490, Copyright © 2006 by the AAP.

Urea cycle intermediates

In AL deficiency, argininosuccinate accumulates and is excreted in the urine. Because argininosuccinate contains two waste nitrogen atoms, production of this metabolite can be exploited to excrete waste nitrogen in AL deficiency, provided that an adequate amount of ornithine is present to supply the necessary carbon skeletons for argininosuccinate biosynthesis. By administering pharmacologic doses of arginine, ornithine is synthesized by the action of arginase. Citrulline and argininosuccinate are then produced in turn by the sequential action of OTC and AS. In AL deficiency, argininosuccinate cannot be further metabolized and is excreted in the urine, along with waste nitrogen (Figure 10–2).[4]

Similarly, as long as sufficient arginine is supplied, citrulline can serve as a vehicle for waste nitrogen excretion in AS deficiency (citrullinemia; Figure 10–2). When compared to argininosuccinate, however, citrulline has two major

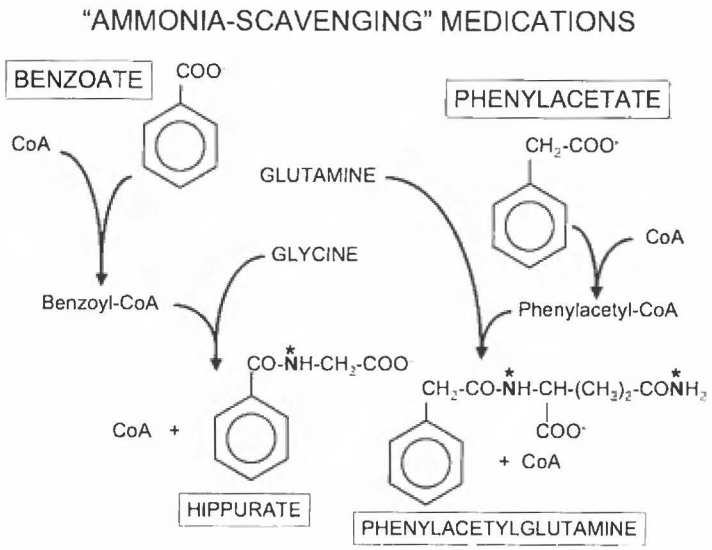

Figure 10–3: Mechanism of nitrogen scavenging by sodium benzoate and sodium phenylacetate: HIP and PAGN are formed by conjugation of benzoate with glycine and phenylacetate with glutamine, respectively. These reactions are performed by specific liver and kidney *N*-acyltransferases. HIP contains one waste nitrogen atom, and phenylacetylglutamine contains two waste nitrogen atoms. Both HIP and PAGN are excreted in the urine, effectively decreasing nitrogen flux through the urea cycle. *Nitrogen atoms excreted. Reproduced with permission from *NeoReviews*, Vol. 7, Page e490, Copyright © 2006 by the AAP.

disadvantages: (1) It contains only one waste nitrogen atom, and (2) a high percentage of filtered citrulline is reabsorbed, so urine excretion is relatively poor.[4,23]

Amino acylation products

Because of high renal clearance (five times the glomerular filtration rate), HIP is easily excreted by the kidneys. HIP biosynthesis, by conjugation of benzoate with glycine, is accomplished by the action of mitochondrial matrix enzymes (benzoyl thiokinase and a glycine-specific *N*-acyltransferase; Figure 10–3). Similarly, PAGN is formed by sequential action of phenylacetyl thiokinase and a glutamine-specific *N*-acyltransferase. Because phenylacetate has the ability to conjugate glutamine, forming PAGN (a compound that contains two waste nitrogen atoms), its nitrogen-scavenging ability was hypothesized to be twice as effective as benzoate (which contains one nitrogen atom).[24]

Brusilow and colleagues suggested using combined therapy with sodium phenylacetate and sodium benzoate (Ammonul; Ucyclyd Pharma, Inc., Scottsdale, AZ) along with intravenous arginine HCl for treating hyperammonemic coma. Ammonul, a combination of sodium phenylacetate (10%) and sodium benzoate (10%), is an intravenously administered drug used as adjunctive therapy for the treatment of acute hyperammonemia and associated encephalopathy in patients with UCDs (Table 10–2).[24] The concomitant use of Ammonul with protein restriction, high caloric nutrition, arginine HCl, adequate hydration, and

Table 10–2. Acute management of patients with UCDs

Table 10–2. Acute management of patients with UCDs

Administration	Components of infusion solution: Ammonul	Arginine HCl injection, 10%	Dextrose injection, 10%	Dosage provided: Sodium phenylacetate	Sodium benzoate	Arginine HCl injection, 10%
Patients weighing 0–20 kg						
			AL deficiency			
Loading dose: over 90–120 min	2.5 mL/kg	6.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	600 mg/kg
Maintenance dose: over 24 h	2.5 mL/kg	6.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	600 mg/kg
			AS deficiency			
Loading dose: over 90–120 min	2.5 mL/kg	6.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	600 mg/kg
Maintenance dose: over 24 h	2.5 mL/kg	6.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	600 mg/kg
			CPS deficiency			
Loading dose: over 90–120 min	2.5 mL/kg	2.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	200 mg/kg
Maintenance dose: over 24 h	2.5 mL/kg	2.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	200 mg/kg
			OTC deficiency			
Loading dose: over 90–120 min	2.5 mL/kg	2.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	200 mg/kg
Maintenance dose: over 24 h	2.5 mL/kg	2.0 mL/kg	25 mL/kg	250 mg/kg	250 mg/kg	200 mg/kg
Patients weighing >20 kg						
			AL deficiency			
Loading dose: over 90–120 min	55 mL/m^2	6.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	600 mg/kg
Maintenance dose: over 24 h	55 mL/m^2	6.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	600 mg/kg
			AS deficiency			
Loading dose: over 90–120 min	55 mL/m^2	6.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	600 mg/kg
Maintenance dose: over 24 h	55 mL/m^2	6.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	600 mg/kg
			CPS deficiency			
Loading dose: over 90–120 min	55 mL/m^2	2.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	200 mg/kg
Maintenance dose: over 24 h	55 mL/m^2	2.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	200 mg/kg
			OTC deficiency			
Loading dose: over 90–120 min	55 mL/m^2	2.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	200 mg/kg
Maintenance dose: over 24 h	55 mL/m^2	2.0 mL/kg	25 mL/kg	5.5 g/m^2	5.5 g/m^2	200 mg/kg

Note: Appropriate dosing of alternative pathway therapy medications for patients weighing up to 20 kg and greater than 20 kg is shown. Arginine HCl may be mixed directly with Ammonul or infused via a separate line. A central line is recommended for delivery of Ammonul and arginine HCl, because serious burns have resulted from extravasation from a peripheral catheter. The loading dose infusion should be given in 10% glucose at 25–35 mL/kg (or in 10% glucose at 400–600 mL/m^2 in older patients). Odansetron may be given (0.15 mg/kg) over the first 15 minutes of the priming infusion to decrease the risk of emesis.[4] Medical management of UCDs has the potential to decrease plasma ammonium levels; however, in cases associated with extreme hyperammonemia, a form of hemodialysis or hemofiltration is typically needed. Although institution of alternative pathway therapy and appropriate caloric support should be started without delay, the possibility of transferring the neonate to a specialist center with hemodialysis capacity should be explored concurrently if the initial point-of-care facility lacks appropriate resources to perform urgent dialysis.

Table 10–3. Chronic management of patients with UCDs

UCD		g/kg/d	g/m²/d
CPS or OTC deficiency	Sodium phenylbutyrate	0.45–0.60	9.9–13.0
	Citrulline or arginine (free base)	0.17	3.8
AS deficiency	Sodium phenylbutyrate	0.45–0.60	9.9–13.0
	Arginine (free base)	0.40–0.70	8.8–15.4
AL deficiency	Arginine (free base)	0.40–0.70	8.8–15.4

Note: Long-term care of UCD patients requires careful evaluation of nutritional status and provision of adequate protein (including essential amino acid formulas), calories, and essential vitamins and nutrients to promote growth, in addition to appropriate medications. The g/kg/d dosing is used for patients weighing up to 20 kg, and the g/m²/d dosing is used for older patients weighing more than 20 kg. Tablets or powder should be taken in equally divided amounts with each meal or feeding (i.e., three to six times daily). Patients should be followed by a center skilled in management of inborn errors of metabolism.

hemodialysis in acute hyperammonemia associated with altered mental status helps to increase waste nitrogen excretion through the formation of HIP and PAGN by the two different pathways discussed earlier in text (Figures 10–1 and 10–2). Hemodialysis is recommended in cases of severe hyperammonemia or if ammonium levels are not significantly reduced within four to eight hours after starting Ammonul.[4]

Sodium phenylbutyrate (Buphenyl; Ucyclyd Pharma, Inc.) is a prodrug of sodium phenylacetate that is used for chronic management of UCDs. Buphenyl, administered enterally in tablet or powder form, is used as adjunctive therapy, along with appropriate dietary management, for outpatient treatment of CPS, OTC, and AS deficiencies (Table 10–3).[4] A clinical trial has started to evaluate whether Buphenyl, in conjunction with decreased arginine dosing, will be beneficial in treating AL deficiency, especially with respect to liver function. Sodium benzoate in powder form is also sometimes used, either alone or in conjunction with Buphenyl, for chronic management of UCD patients.

Pharmacokinetics of Ammonul

Both phenylacetate and benzoate demonstrate saturable, nonlinear elimination, with a decrease in clearance with increased dose. Therefore, following established treatment protocol dosing guidelines is important to avoid an overdose. A second bolus infusion is not recommended if plasma ammonium levels do not drop significantly after the initial bolus.[4] Brusilow and colleagues studied the pharmacokinetics of intravenous sodium phenylacetate and sodium benzoate in two children (five months old and one year old) with CPS deficiency. The peak levels of phenylacetate and benzoate occurred at the same time (approximately two to three hours following dosing), and the benzoate level decreased faster. Phenylacetate levels were initially higher than benzoate levels and remained so throughout the period of study. HIP reached a peak earlier than PAGN but PAGN levels remained high for a longer period when compared to HIP in both patients. Urinary HIP nitrogen (18–57% of waste nitrogen) and urinary PAGN

nitrogen (15–53% of waste nitrogen) combined accounted for approximately 60% of "effective" urinary waste nitrogen.[25]

There are no published pharmacokinetic studies on Ammonul performed exclusively in neonates. Green and colleagues, however, monitored the disposition of intravenous sodium benzoate alone in hyperammonemic newborn infants (n = 4) following administration of 460 mg/kg/d in four divided doses. An eightfold range in serum benzoate concentrations between treated neonates was noted. The elimination half-life of benzoate was 2.8 ± 3.1 hours. The total plasma clearance of benzoate was 1.00 ± 0.61 mg/kg/min, the majority being attributed to glycine conjugation in three of four neonates. The excreted total of benzoate and HIP was 84% (±31%) of the administered benzoate. One neonate with reduced renal clearance excreted only 12% of benzoate as HIP. In this case, PD was the major route of benzoate clearance.[26] Intravenous infusions of benzoate and phenylacetate (given on different days) were administered to five children with lysinuric protein intolerance who were clinically stable during the period of study. Plasma benzoate levels peaked at 6.0 mmol/L (range 5.2–7.0 mmol/L) two hours after the start of the infusion (2.0 mmol/kg over 90 minutes) and decreased linearly with a mean half-life of 273 minutes. Plasma HIP levels peaked 120 minutes after the start of the infusion at 0.24 mmol/L (range 0.14–0.40 mmol/Lm) and remained stable for three hours. Less than 2% of the administered dose of benzoate appeared unchanged in the urine. Plasma phenylacetate levels also peaked at 120 minutes and decreased similarly to benzoate ($t_{1/2}$ = 253 minutes), although peak levels were lower (4.8 mmol/L, range 3.7–6.1 mmol/L). Plasma PAGN levels peaked at 270 minutes with a mean concentration of 0.48 mmol/L (range 0.22–1.06 mmol/L). Forty percent (range 15–110%) of infused phenylacetate was excreted as PAGN in 24 hours.[27]

Both sodium phenylacetate and sodium benzoate are low-molecular-weight molecules with poor protein binding. Hemodialysis and hemofiltration result in significant dialytic and convective clearances of Ammonul, respectively, although therapeutic plasma levels of Ammonul may still be obtained despite high extracorporeal clearance.[28]

DOSAGE

Infants and young children (weighing up to 20 kg) with CPS deficiency, OTC deficiency, or AS deficiency should be treated with Ammunol at a load of 250 mg/kg intravenously over a period of 90–120 minutes. Older children and adults (weighing more than 20 kg) should be treated with Ammonul at 5.5 g/m^2 as an intravenous load over a period of 90–120 minutes (Table 10–2). After the loading dose, maintenance infusions of the same dose over 24 hours may be continued until the patient is no longer hyperammonemic and oral therapy can be tolerated.[4,8] Specific guidelines for administering Ammonul are not provided for treatment of AL deficiency or arginase deficiency. AL deficiency may respond to intravenous

arginine HCl alone (although other interventions, including Ammonul use and hemodialysis, are needed in some cases), and arginase deficiency has only rarely been associated with significant hyperammonemia. Loading and maintenance infusions should contain arginine HCl (210 mg/kg for patients with OTC or CPS deficiency or 630 mg/kg for patients with AS or AL deficiency). Ammonul is diluted in sterile 10% dextrose in both loading and maintenance infusions. Ten percent arginine HCl can be mixed in the same dextrose solution as Ammonul. Because of the saturable pharmacokinetics of phenylacetate, no more than one loading dose of Ammonul is recommended regardless of the initial level of plasma ammonium. The maintenance infusion can be continued until plasma ammonium levels are within normal limits.[4] Ammonul should be administered through a central line because extravasation may cause irritation, burns, and necrosis.

Appropriate nutrition, temporary protein restriction, and adequate hydration must also be used in conjunction with Ammonul and arginine HCl to maximize clearance of plasma ammonium. Hemodialysis and/or continuous hemofiltration should be started without delay if alternative pathway therapy and other interventions do not result in adequate control of plasma ammonium levels. Central access is critical to provide high-dextrose fluids and fat emulsion (Intralipid), with the goal of administering approximately 100–120 kcal/kg/d to neonates. An insulin drip is often needed to control hyperglycemia and promote anabolism.[29] In older patients and adults, a nutritional regimen providing 80 kcal/kg/d is a reasonable initial goal.[7] Protein should be withdrawn immediately and then slowly reintroduced after 24–48 hours.[29]

SIDE EFFECTS

Because of the difficulty in distinguishing symptoms related to hyperammonemia from symptoms caused by a reaction to medication, side effects are similarly difficult to attribute directly to alternative pathway therapy. Oral benzoate therapy has been associated with nausea and vomiting, but overall toxicity appears to be low as long as standard dosing guidelines are followed.[30] The use of benzyl alcohol as a bacteriostatic agent in neonatal intensive care units has resulted in severe metabolic acidosis, lethargy progressing to coma, seizures, and death. Benzoate and HIP, breakdown products of benzyl alcohol, were identified in the urine of these neonates.[31] A theoretical concern related to benzoate use in neonates is its potential ability to displace bilirubin from high-affinity albumin binding sites. There are no known cases of significant hyperbilirubinemia or kernicterus attributable to benzoate use, however.

No side effects, other than an unpleasant odor, were reported in normal human subjects and two patients with urea cycle defects receiving between 1 and 10 grams of phenylacetate.[32] Buphenyl use is associated with body odor caused by the metabolite phenylacetate, and patients have reported an aversion to the medication because of its bad taste. Abdominal discomfort and gastritis may also occur.

The most common adverse reaction reported to be associated with Ammonul is vomiting, occurring in approximately 9% of patients.[33] In a study of healthy adults, MacArthur and colleagues reported nausea, vomiting, and somnolence following administration of Ammonul in doses used to treat hyperammonemia.[34] In a report documenting responses to Ammonul in 299 UCD patients, adverse events were reported in a little more than 50% of treated patients. Most adverse events were likely related to the underlying primary disease or patient clinical status and were reported during treatment for hyperammonemia. Metabolic (hypokalemia, hyperammonemia, hyperglycemia, acidosis), nervous system (seizures, cerebral edema, mental impairment), and respiratory system abnormalities (respiratory distress or failure, hyperventilation) were reported most frequently and occurred in 22%, 18%, and 14% of patients, respectively.[8]

The most significant side effects and toxicity related to Ammonul use have occurred in cases of inadvertent overdose. Continuous intravenous infusion rates that result in plasma phenylacetate levels that saturate the capacity of conversion of phenylacetate to PAGN result in rapid phenylacetate accumulation and subsequent toxicity. Three patients (two to six years old) who were given inappropriately high doses of intravenous Ammonul (915 mg/kg over 12 hours, 1,750 mg/kg over 18 hours, and 750 mg/kg over 10 hours) had plasma benzoate and phenylacetate levels of approximately 10 mmol/L four hours after infusion and developed altered mental status, Kussmaul breathing, metabolic acidosis, cerebral edema, and hypotension. Two of the three patients died; one survived after hemodialysis.[35] Other signs of intoxication with Ammonul include hypernatremia, hyperosmolarity, and cardiovascular collapse.[4] Clearly written medical prescriptions and cross-checking of drug dosage are important safeguards.

U.S. FOOD AND DRUG ADMINISTRATION STATUS

Ammonul received U.S. Food and Drug Administration (FDA) new drug approval on February 17, 2005. Data collected over approximately 25 years of clinical investigation by a multitude of investigators throughout the country was used as a basis for the FDA decision. Ammonul is labeled a Category C drug, so it is not known whether Ammonul can cause harm to the fetus when administered to a pregnant woman or if reproduction capacity can be affected. Therefore, Ammonul use in pregnancy is recommended only if the medication is clearly needed. Caution should also be exercised if Ammonul is administered to pregnant women because it is unknown whether excretion in breast milk occurs. Buphenyl received FDA new drug approval on April 30, 1996 and is also a Category C drug.

RESULTS OF THERAPY

Treatment with Ammonul results in decreased plasma ammonium levels and improved neurological status in most cases, although, if severe hyperammonemia

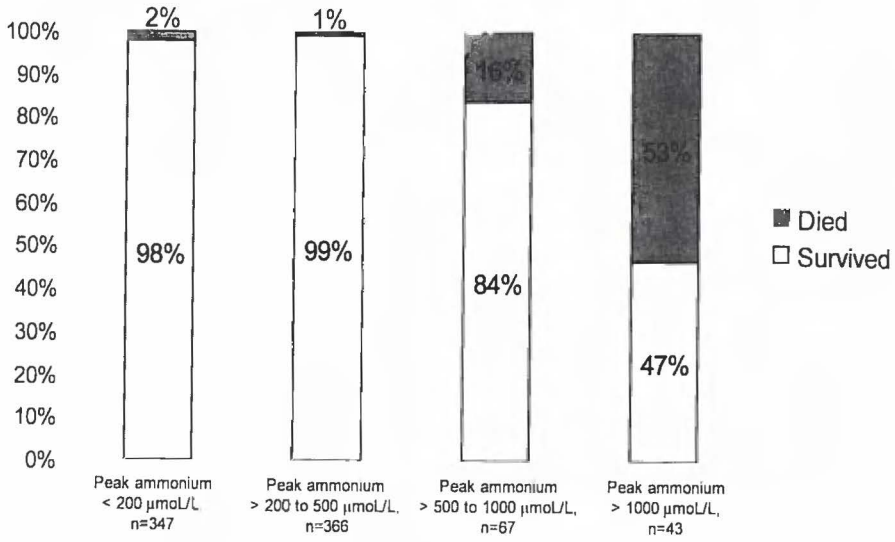

Figure 10–4: Hyperammonemic episodes survived according to peak ammonium levels. Patient survival of hyperammonemic episodes depends on peak plasma ammonium level, with significantly improved survival for patients who experience hyperammonemic episodes with a peak plasma ammonium level of up to 500 µM ($p < 0.001$). Neonates with a peak plasma ammonium level greater than 1,000 µM are least likely to survive a hyperammonemic episode (survival, 38%; $p < 0.001$).[8] From Enns et al. Survival after Treatment with Phenylacetate and Benzoate for Urea Cycle Disorder, *The New England Journal of Medicine*, F2. 2007, Massachusetts Medical Society. All rights reserved.

is present, alternative pathway therapy may be insufficient to have any appreciable effect. Early reports of alternative pathway therapy use in relatively small cohorts documented improved survival compared to that in historical controls.[23,25,26,46] A large study reported the outcome of 299 UCD patients who were treated with Ammonul. Patients sustained 1,181 episodes of hyperammonemia over a 25-year period with an episode survival rate of 96% (neonates: 73%; patients older than 30 days: 98%) and overall patient survival of 84%, a clear improvement when compared to historical data. Survival was also related to the peak plasma ammonium level and patient age. Nearly all episodes in which the ammonium level did not rise above 500 µmol/L resulted in survival, with survival decreasing as ammonium levels increased (Figure 10–4).[8] Because patients were primarily treated by metabolic centers with experience in caring for acute hyperammonemia caused by UCDs, the high survival rate in this study may in part reflect the expertise available at treating institutions. The improved outcome following use of alternative pathway medication is also apparent when comparison is made to outcome data detailed in a European report of 217 UCD patients who did not receive alternative pathway therapy for acute management of hyperammonemia. Only 16% of patients with neonatal-onset disease survived overall, and patient survival in late-onset disease was 72%.[6]

Although alternative pathway therapy in conjunction with other therapies, especially hemodialysis, has led to improved UCD patient survival, cognitive impairment remains a common finding, especially in patients who have

neonatal-onset disease. The age at which the first symptom is noted, however, is not necessarily predictive of outcome in individual cases, because patients who have neonatal-onset disease may still have a normal long-term outcome. A study of 26 children who survived neonatal hyperammonemia was discouraging with respect to neurological outcome; the overwhelming majority (79%) had one or more developmental disabilities at 12–74 months of age. IQ correlated with the depth of coma, but not the peak plasma ammonium level over a range of 351–1,800 μmol/L.[33]

Other studies have found a correlation between the peak plasma ammonium level and cognitive outcome, although Ammonul was not used in these reports. When the concentration of plasma ammonium exceeded 350 μmol/L at the time of the first episode of hyperammonemia, patients either died or had severe neurological deficits in a Japanese study of 108 UCD patients.[37] In a European questionnaire study, no surviving UCD patients with an initial plasma ammonium level greater than 300 μmol/L or a peak plasma ammonium level greater than 480 μmol/L had normal psychomotor development.[38]

Neonatal-onset OTC deficiency is particularly devastating with respect to neurological outcome. Prospective neonatal therapy following prenatal diagnosis by deoxyribonucleic acid (DNA) or biochemical analysis, however, decreases the risk of neonatal hyperammonemia. Infants at risk for hyperammonemia caused by UCDs based on family history may be treated with Ammonul prospectively to prevent hyperammonemic coma. If such proactive therapy is instituted, neonates have a more favorable outcome compared to patients who are rescued from hyperammonemic coma. The brief period of prospective treatment while confirmatory diagnostic studies are pending does not appear to have any adverse effect on the growth and development of those infants who turn out to be unaffected.[36]

The use of Ammonul to treat other conditions that can cause neonatal hyperammonemia, such as organic acidemias, fatty acid oxidation disorders, and transient hyperammonemia of the newborn, has not been studied in detail. These conditions may be difficult to distinguish from UCDs on initial presentation in some instances. Clinicians have used Ammonul to treat non-UCD conditions, such as organic acidemias or transient hyperammonemia of the newborn, with variable efficacy.

FUTURE DEVELOPMENTS

The National Institutes of Health has sponsored the formation of a Rare Disease Clinical Research Center network for UCDs. This establishment of a network of specialized centers with expertise in providing state-of-the-art treatment for metabolic disorders has the potential to improve neurologic outcomes. A multicenter longitudinal study has been initiated and has already provided new information about the natural history of these disorders.[39] The prospective treatment of children at risk for hyperammonemic coma and other therapeutic modalities, especially liver transplantation, also play significant roles in the management of

patients with UCDs and may improve outcome. Although hepatocyte transplantation is a promising new approach for the treatment of liver-based metabolic disorders, only limited success has been reported to date.[40] Gene therapy also holds promise for the treatment of these disorders, but significant technical hurdles need to be overcome, as is clear following the fatal occurrence of systemic inflammatory response syndrome in an OTC patient following adenoviral gene transfer.[41] Until the aforementioned technologies can be developed for wider application, liver transplantation is currently the only definitive therapy for these patients.

A new oral medication is being developed for hyperammonemia control and is currently in clinical trials. Glycerol phenylbutyrate [glyceryl tri-(4-phenylbutyrate)] is an investigational agent that is a prodrug of sodium phenylbutyrate (currently marketed as Buphenyl). Like sodium phenylbutyrate, glycerol phenylbutyrate is metabolized in the liver to phenylacetate, which in turn conjugates to glutamine and forms PAGN. Glycerol phenylbutyrate is a triglyceride containing three molecules of 4-phenylbutyric acid joined via ester linkage to glycerol. Glycerol phenylbutyrate is an organic liquid (oil) with little odor or taste that delivers the same amount of phenylbutyrate in a compact form (e.g., ~17.4 milliliters of glycerol phenylbutyrate [a little more than one teaspoon three times daily] delivers the same amount of phenylbutyric acid as do 40 tablets of sodium phenylbutyrate). Pharmacological data from Cynomolgus monkeys, which have the capacity to metabolize phenylacetate to form PAGN, suggest that glycerol phenylbutyrate acts as a slow-release product which may be converted to PAGN more efficiently than sodium phenylbutyrate.[42] Ten adult UCD subjects were switched to glycerol phenylbutyrate from sodium phenylbutyrate. Compared to treatment with sodium phenylbutyrate, glycerol phenylbutyrate treatment resulted in approximately 30% lower plasma ammonium values (as assessed by time-normalized area under the curve, findings not statistically significant), similar plasma PAGN and amino acid levels, and similar urinary excretion of PAGN. Somewhat fewer adverse events were reported during the glycerol phenylbutyrate period of this trial (21 adverse events in seven subjects during sodium phenylbutyrate treatment compared to 15 adverse events in five subjects during glycerol phenylbutyrate treatment), and the only two hyperammonemic events during this study occurred in subjects on sodium phenylbutyrate.[43] Given the difficulties facing urea cycle patients with respect to daily pill burden and the noxious taste of sodium phenylbutyrate, glycerol phenylbutyrate has the potential to improve compliance and chronic plasma ammonium control if the promise of the initial small trial results is confirmed in larger-scale studies.

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