Alternative pathway therapy for urea cycle disorders: Twenty years later

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Alternative pathway therapy is currently an accepted treatment approach for inborn errors of the urea cycle. This involves the long-term use of oral sodium phenylbutyrate, arginine supplements, or both, depending on the specific enzyme deficiency, and treatment of acute hyperammonemic crises with intravenous sodium benzoate/sodium phenylacetate plus arginine. A review of 20 years of experience with this approach illustrates the strengths and limitations of this treatment. It has clearly decreased the mortality and morbidity from these disorders, but they remain unacceptably high. The medications are generally well tolerated, but severe accidental overdosage has been reported because of the infrequent use of the medication. There is also a difference in their metabolism between newborns and older children that must be addressed in determining dosage. To avoid these complications it is recommended that drug levels in blood be monitored routinely and that very specific treatment protocols and oversight be followed to avoid overdoses. Finally, it must be acknowledged that alternative pathway therapy has limited effectiveness in preventing hyperammonemia and must be combined with effective dietary management. Therefore in children with neonatal-onset disease or in those with very poor metabolic control, liver transplantation should be considered. There should also be the continued search for innovative therapies that may offer a more permanent and complete correction, such as gene therapy. (J Pediatr 2001;138:S46-S55)

The idea of using a detour around a congenital block within the urea cycle was the result of a serendipitous observation that occurred 20 years after these disorders were first discovered[1] and at a time when they carried a universally fatal prognosis.[2] Although alternative pathway therapy has led to significant improvements in mortality and morbidity, half of the children who survive neonatal-onset ornithine transcarbamylase or carbamyl phosphate synthetase deficiency still die before entering school,[3] and those who survive have a high incidence of developmental disabilities.[4] This has led to the increased use of liver transplantation in treating patients with neonatal-onset disease.[5] It has also resulted in a search for the next generation of treatment, potentially gene therapy.[6] However, until liver transplantation becomes more widely accessible or gene therapy technically

ASA	Argininosuccinic acid
ASL	Argininosuccinic acid lyase
ASS	Argininosuccinic acid synthetase
CPS	Carbamyl phosphate synthetase I
OTC	Ornithine transcarbamylase
PA	Phenylacetate
PAG	Phenylacetylglutamine
PB	Phenylbutyrate
PKU	Phenylketonuria

feasible, alternative pathway therapy combined with protein restriction will remain the mainstay of therapy for inborn errors of urea synthesis. Thus it is useful to review the first 2 decades of experience with this approach, discuss the benefits and risks, and develop guidelines for best practice in the treatment of hyperammonemic crises and in the chronic management of these disorders.

THE SERENDIPITY OF DISCOVERY

The word serendipity was coined by Horace Walpole after the characters in the fairy tale "The Three Princes of Serendip," who made fortunate and unexpected discoveries by accident.[7] In the case of alternative pathway therapy, a serendipitous observation was made by Dr Saul Brusilow. In

re-examining the data in an article on ketoacid therapy for citrullinemia (argininosuccinic acid synthetase deficiency) that we had previously published,[8] he noted a previously unrecognized pattern; the more arginine that was given to the patient, the more nitrogen was excreted in the urine, and the lower the blood ammonia level was. He hypothesized that arginine was stimulating an alternative pathway for waste nitrogen excretion through increasing the synthesis of citrulline that contained a nitrogen from ammonia and could be rapidly excreted in the urine.[9] Shortly thereafter, we had the opportunity of testing this theory on a newborn infant with argininosuccinic aciduria (argininosuccinic acid lyase deficiency), in whom the same principle would apply. In this case, with argininosuccinic acid as the waste nitrogen product, the provision of 4 mmol/kg (800 mg/kg) of arginine hydrochloride intravenously was associated with a remarkable and rapid fall in ammonia.[10] The use of arginine to stimulate an alternative pathway for waste nitrogen excretion has remained a mainstay of therapy for these 2 disorders to the present day.

A subsequent serendipitous observation led to the use of sodium benzoate, sodium phenylacetate, and sodium phenylbutyrate. Dr Norman Radin, a biochemist from the University of Michigan, was a National Institutes of Health site visitor for the Johns Hopkins Clinical Research Center in 1978, where we presented our research. We discussed the use of arginine to stimulate the urea cycle flux in argininosuccinic aciduria, and Dr Radin remarked that he remembered reading an article from around 1914 in the *Journal of Biological Chemistry* that spoke of using sodium benzoate to stimulate hippurate synthesis and decrease urea nitrogen excretion. After the site visit we went down to the bowels of the Welch medical library and found the article[11] and a second article published a few years later that spoke of using sodium phenylacetate in a similar vein.[12] Such was the birth of alternative pathway therapy for urea cycle disorders.[13]

PHARMACOLOGY OF ALTERNATIVE PATHWAY THERAPY

In the subsequent 2 decades much has been learned about the pharmacology of these compounds and their efficacy in treating patients with acute and chronic hyperammonemia. Sodium benzoate, by its acylation of glycine to form hippurate and its renal clearance being fivefold the glomerular filtration rate, has the potential of removing 1 mole of waste nitrogen for each mole of benzoate administered. This conversion occurs primarily in the liver and kidney by glycine N-acyltransferase.[14] Sodium phenylacetate is conjugated with glutamine by the enzyme phenylacetyl CoA:glutamine acyltransferase, to form phenylacetylglutamine, which is excreted by the kidney. This conversion also occurs in the liver and kidney.[15] Glutamine contains 2 waste nitrogen atoms, so 2 moles of nitrogen could be removed for each mole of phenylacetate administered. Sodium benzoate and sodium phenylacetate remain useful in their intravenous formulation for the treatment of patients with acute hyperammonemia. Sodium phenylbutyrate (Buphenyl, Ucyclyd Pharma),[16] which is first activated to its CoA ester, then metabolized by β-oxidation in the liver to phenylacetyl-CoA, and subsequently conjugated with glutamine, has replaced these compounds for chronic oral use. It has the advantage of lacking the disagreeable odor of the phenylacetate while maintaining its efficient nitrogen removal. For chronic oral use in patients who do not respond to or are intolerant of sodium phenylbutyrate, sodium phenylacetate with sodium benzoate oral liquid is also available from compounding pharmacies. This compound was previously marketed under the brand name of Ucephan.

Intravenous Sodium Benzoate

Green et al[17] studied the pharmacokinetics of intravenous sodium benzoate in 4 neonates in hyperammonemic coma, 3 caused by OTC deficiency. With a dose of 3.5 mmol/kg/d in 4 divided doses (460 mg/kg/d), a mean of 84% of administered benzoate was found to be excreted as benzoate and hippurate in the urine. Serum benzoate concentrations ranged from 2.1 to 16 mmol/L. Three of the 4 neonates cleared >50% of benzoate by the metabolic route (glycine conjugation). In these patients benzoate was not removed efficiently enough by peritoneal dialysis to prevent hippurate conversion, presumably because it is protein bound. Under these circumstances the addition of peritoneal dialysis was synergistic with benzoate administration in the removal of ammonia. One neonate excreted only 12% of benzoate as hippurate. In this patient peritoneal dialysis served as the primary route of benzoate excretion. This may have been related to reduced renal clearance (caused by hypotension and impaired renal function) or limited benzoate conjugation capacity.

Simell et al[18] studied intravenous administration of sodium benzoate in 5 older children with lysinuric protein intolerance who were clinically stable at the time of the study. Plasma benzoate levels peaked 2 hours after an infusion of 2 mmol/kg was started and had a half-life of 273 minutes. The mean plasma benzoate level at the end of the 90-minute infusion was 6 mmol/L (range 5.2 to 7.0 mmol/L). Plasma hippurate peaked at 120 minutes at 0.24 mmol/L (0.14 to 0.4 mmol/L) and remained stable for the subsequent 3 hours. Less than 2% of administered sodium benzoate was excreted unchanged in urine over 24

hours; hippurate excretion peaked in the first 6 hours, declining steadily thereafter. Total hippurate excretion accounted for 67% (35% to 126%) of benzoate administered.

These studies suggest that sodium benzoate is useful for the acute treatment of patients with hyperammonemia. It is rapidly metabolized and removes significant amounts of nitrogen.[19,20] However, there are limitations; the response is stoichiometric, with a maximum of 1 mole of nitrogen removed for each mole of benzoate administered, and the dosage is limited by potential toxicity. Thus when there is massive and incremental nitrogen accumulation, as during hyperammonemic coma (plasma ammonia >250 µmol/L), benzoate treatment will be insufficient, even when combined with sodium phenylacetate. However, it may well have a synergistic effect with dialysis. The wide variability of its metabolism in infants and neonates may result from immaturity of the acylation system in the liver and kidney. Between birth and 7 months of age, hepatic mitochondrial glycine N-acyltransferase activity has been shown to vary from 5% to 40% of the peak activity, which occurs by 18 months of age.[21] This emphasizes the importance of monitoring plasma benzoate levels to avoid toxicity.

Intravenous Sodium Phenylacetate

Thibault et al[22] studied the pharmacokinetics of intravenous PA in adults with cancer. PA was administered as a bolus dose of 0.8 mmol/kg (150 mg/kg) followed by a continuous infusion. Serum PA levels displayed nonlinear pharmacokinetics. These authors individualized each patient's continuous infusion dose based on parameters derived from the initial bolus infusion. No toxicity was associated with bolus administration of PA. During the continuous infusion the authors were able to achieve stable phenylacetate concentrations of 1 to 1.6 mmol/L, although induction of clearance and

nonlinear kinetics made this difficult in some cases. Plasma PA concentrations of 5 to 7 mmol/L were associated with dose-limiting toxicity of reversible central nervous system depression, preceded by emesis. Simultaneous serum and cerebrospinal fluid sampling showed comparable phenylacetate levels and the presence of phenylacetylglutamine in the cerebrospinal fluid. This result suggests that PA can hasten the decline in glutamine and thereby ammonia from brain tissue, providing glutamine N-acylase is expressed in brain.

In a subsequent study the authors used 2 daily bolus doses of 125 and 150 mg/kg per dose, rather than a continuous infusion, to achieve the targeted plasma levels. Except for 1 occurrence, all cases of dose-limiting toxicity were seen at the 150 mg/kg per dose and were neurologic in nature. The peak PA levels associated with toxicity ranged from 2.7 to 5.5 mmol/L.

Simell et al[18] studied the use of intravenous PA in lysinuric protein intolerance. Plasma PA levels 120 minutes after infusion of 2 mmol/kg (375 mg/kg) were less than plasma benzoate after an equimolar infusion, but the half-life of 254 minutes was similar. The plasma PA level was 4.8 mmol/L (range 3.7 to 6.1 mmol/L) at the end of the infusion. Plasma PAG peaked at 270 minutes at 0.48 mmol/L (range 0.22 to 1.06 mmol/L), a level above that seen for hippurate after an equimolar infusion of sodium benzoate. Forty percent (range 15% to 110%) of infused PA was excreted as PAG in 24 hours, mostly within 12 hours. Peak plasma PA levels of 4 mmol/L were not associated with adverse clinical symptoms.

Intravenous Sodium Benzoate and PA in Combination

Brusilow et al[23] reported on the efficacy of the combination of intravenous sodium benzoate and PA in the management of intercurrent hyperammonemic crises in children with urea cycle disorders. The dose used was 250 mg/kg of each, given as a bolus over a

2-hour period followed by a continuous infusion of 250 mg/kg per 24 hours until the ammonia level had stabilized. Eleven of 12 episodes were treated successfully, and measurement of the distribution of urinary nitrogen revealed that nitrogen in hippurate and PAG together accounted for an average of 60% of urinary waste nitrogen excretion. In 1 patient drug and metabolite plasma levels were described after a bolus and continuous infusion. For PA, maximal levels of 3 to 4 mmol/L were achieved after the bolus dose and remained within that range for approximately 20 hours. PAG levels were not detected for 10 hours after the infusion, although the conjugation of phenylacetate is known to occur rapidly.[24] This was probably due to assay limitations. Plasma benzoate levels peaked at approximately 2.5 mmol/L after the bolus infusion and then descended to <1 mmol/L by approximately 15 hours. Hippurate elevations were immediately detectable in plasma and remained so during the continuous infusion, in the range of 0.2 to 0.4 mmol/L.

Sodium Phenylbutyrate

In studying the intravenous pharmacokinetics of sodium phenylbutyrate in adults, Piscitelli et al[24] used doses of 600 to 2000 mg/m^2 (equivalent to doses of approximately 25 to 90 mg/kg in a child) given intravenously over a 30-minute period. Peak plasma PB concentrations ranged between 0.17 and 0.30 mmol/L after the 600 mg/m^2 dose, between 0.3 and 0.6 mmol/L after the 1200 mg/m^2 dose, and between 0.6 and 1.0 mmol/L after the 2000 mg/m^2 dose. By 5 hours after the dose, levels were barely detectable in all cases, indicating first-order elimination. Eighty percent of PB appeared in the urine as PAG. PA was detected in the urine within 10 minutes of the start of the infusion, with a peak concentration 30 to 60 minutes after completion of the infusion of 0.13 mmol/L, but levels were lower than when PA itself is infused. PA lev-

Table I. Use of alternative pathway therapy during intermittent hyperammonemic crisis in patients with urea cycle disorders*

Disorder	Drug administration	Sodium benzoate	Sodium phenylacetate	10% Arginine HCl
CPS or OTC deficiency	Priming infusion	0.250 g/kg or 5.5 g/m^2	0.250 g/kg or 5.5 g/m^2	0.20 g/kg (2 mL/kg) or 4.0 g/m^2
	Sustaining infusion	0.250 g/kg/24 h or 5.5 g/m^2/24 h	0.250 g/kg/24 h or 5.5 g/m^2/24 h	0.20 g/kg (2 mL/kg)/24 h or 4.0 g/m^2/24 h
Argininosuccinic acid synthetase deficiency	Priming infusion	0.250 g/kg or 5.5 g/m^2	0.250 g/kg or 5.5 g/m^2	0.60 g/kg (6 mL/kg) or 12.0 g/m^2
	Sustaining infusion	0.250 g/kg/24 h or 5.5 g/m^2/24 h	0.250 g/kg/24 h or 5.5 g/m^2/24 h	0.60 g/kg (6 mL/kg)/24 h or 12.0 g/m^2/24 h
Argininosuccinic acid lyase deficiency	Priming infusion	—	—	0.60 g/kg (6 mL/kg) or 12.0 g/m^2
	Sustaining infusion	—	—	0.60 g/kg (6 ml/kg)/24 h or 12.0 g/m^2/24 h
Arginase deficiency	Priming infusion	0.250 g/kg or 5.5 g/m^2	0.250 g/kg or 5.5 g/m^2	—
	Sustaining infusion	0.250 g/kg/24 h or 5.5 g/m^2/24 h	0.250 g/kg/24 h or 5.5 g/m^2/24 h	—

*If the patient is already on therapy, primary infusion dose should be reduced or eliminated.

els in plasma were also much lower (below Km) after intravenous PB than after PA infusion.

Brusilow[25] noted that children receiving a diet containing 200 mg/kg/d nitrogen (1.25 g/kg/d protein) excrete 94 mg urea nitrogen/kg/d, 47% of dietary nitrogen. To excrete this amount of PAG nitrogen he calculated that the child would require 525 mg/kg/d PA provided as phenylbutyrate. He subsequently found that 80% to 90% of the predicted amount of PAG synthesized was excreted. In children with urea cycle disorders on a low-protein diet, PB administered orally at the recommended dose of 300 to 650 mg/kg/d yielded plasma levels of PA ranging from 0.026 to 1.87, of PB ranging from 0 to 0.872, and of PAG ranging from 0.093 to 3.15 mmol/L.

Arginine

Arginine free base is used as a long-term alternative pathway therapy to treat patients with ASS and ASL deficiencies at a dose of 3 to 4 mmol/kg/d (500 to 700 mg/kg/d).[26] This dose has been well tolerated and is associated with plasma arginine levels 1.5-fold to twofold normal (mean 128 μmol/L). It also leads to further increases in plas-ma levels of citrulline (mean 3936 μmol/L) and ASA (907 μmol/L), respectively, which are already markedly elevated in these disorders. There is significant excretion of citrulline and ASA in urine, representing 33% to 37% of waste nitrogen excretion in ASS deficiency and 52% to 59% in ASL deficiency. In ASL deficiency, arginine therapy combined with protein restriction has proven very effective for long-term control; in ASS deficiency the combination of arginine and phenylbutyrate has been effective.[27,28]

ADVERSE EFFECTS OF ALTERNATIVE PATHWAY THERAPY

Animal studies have suggested potential toxicities from benzoate administration as a result of its completion for free CoA[29] and impairment of mitochondrial pathways and glycine production.[30,31] In addition, benzoate can theoretically displace bilirubin from high-affinity albumin binding sites. However, sodium benzoate and other alternative pathway therapies have been found to be remarkably nontoxic in humans at the doses recommended to treat patients with urea cycle disorders. Laboratory studies of hematopoietic, renal, and hepatic function have been within normal limits, and pathologic examination of the liver revealed normal hepatic tissue except for a small amount of fibrosis,[26] a finding similar to patients not receiving alternative pathway therapy.[32]

During long-term PB therapy, 23% of menstruating females had irregular menses or became amenorrheic. Decreased appetite, taste disturbances, or disagreeable body odor occurred in approximately 4%. Abnormal electrolytes, increased serum hepatic enzyme levels, hypoalbuminemia, and anemia may occur but are difficult to differentiate from the primary disease itself. A variety of gastrointestinal disorders, aplastic anemia, eccymoses, arrhythmias, renal tubular acidosis, depression, and rash have been reported rarely.[33,34] Renal Fanconi syndrome has been reported in 2 patients, and a few patients were reported to have oral mucositis[35]; there has been 1 report of chronic pancreatitis.[35a] Neurotoxicity as described in adults receiving PA is unlikely to occur, because PA levels remain low after PB administration.[24]

In terms of side effects of the intravenous bolus doses of sodium benzoate and PA used to treat patients with hyperammonemic crises (Table I), there is an association with nausea and vomiting during the infusion and with hypokalemia related to urinary loss that is enhanced by the excretion of the nonabsorbable hippurate and PAG. In children severe toxicity of intravenous benzoate and phenylacetate is rare, but overdoses (3 to 5 times normal doses) have led to symptoms that mimic hyperammonemic crises including agitation, confusion, and hyperventilation. Plasma ammonia levels may not be elevated initially, but there is often subsequent rebound hyperammonemia. There was also a partially compensated metabolic acidosis and an increased anion gap (up to 52 meq/L). Two deaths caused by drug overdose have been reported; these were associated with cerebral edema, hypotension, and cardiovascular collapse.[26,36] The overdosage ranged from 750 mg/kg administered over a 10-hour period to 1750 mg/kg over an 18-hour period. These children had plasma benzoate and phenylacetate levels of approximately 10 mmol/L 4 hours after infusion. This disproportionate increase in plasma levels is attributable to the nonlinear pharmacokinetics of phenylacetate. In adults plasma PA levels of >6 mmol/L were associated with confusion, lethargy, and emesis.[37]

In children with ASS and ASL deficiency, at the recommended bolus dose of arginine hydrochloride (3 to 4 mmol/kg/d), severe adverse events have not been reported, although hyperchloremic acidosis has been noted, and extravasation of arginine hydrochloride can cause tissue necrosis. A 21-month-old girl with short stature who was inadvertently given 18.5 mmol/kg (3.9 g/kg; 300 mL of 10% arginine hydrochloride solution intravenously) as a bolus to measure growth hormone stimulation had a fatal reaction.[38] Within 30 minutes of the infusion she had gasping respiration and a cardiopulmonary arrest. She was re-

suscitated but had suffered irreversible brain damage and died a few days later. She was found to be acutely hyponatremic with a metabolic acidosis; the arrest was thought to have developed from an arrhythmia caused by the sudden drop in pH.

There is no proven toxicity from chronic therapy with arginine-free base, but there are some unresolved issues. One is that children with ASL deficiency have markedly enlarged livers, and in some cases there is evidence of cirrhosis.[32] Because arginine treatment leads to increases in both arginine and ASA in blood, there is the concern that it could potentially contribute to the hepatopathology. However, a review of patients treated before the use of arginine reveals hepatomegaly with fibrosis; thus it is unclear whether arginine is a contributing factor.[39] The second concern is whether increasing citrulline or ASA levels in blood, and presumably brain, could have an adverse effect on intellectual function. Msall et al[40] followed a few patients with ASS or ASL deficiency over time while they were receiving arginine therapy and found stable IQ scores over a number of years; however, these children all had pre-existing mental retardation resulting from neonatal hyperammonemic coma. An approach to decreasing the levels of arginine, citrulline, and ASA could be to use a combination of phenylbutyrate and arginine therapy in both ASS and ASL deficiencies.

OUTCOME MEASURES

Although there have been no controlled studies, introduction of alternative pathway therapy appears to have improved both biochemical control and neurologic outcome in patients with urea cycle disorders.[4,26-28,41-43] There has been a reported decrease in the frequency of episodes of hyperammonemia, improved growth, and maintenance of intellectual function. Children with neonatal-onset ASS and ASL deficien-

cies have better survival rates than those with OTC and CPS deficiency. The vast majority of both groups, however, have significant developmental disabilities.[4,44] The exception has been children who were treated prospectively rather than having been rescued from hyperammonemic coma.[42] These children, however, remain at risk for potentially fatal hyperammonemic coma throughout their lives, and to our knowledge no patient with neonatal onset of CPS or OTC deficiency has yet survived to adulthood. For this reason it is now advised that these children be considered for liver transplantation in their first year of life.

For children with late-onset disease (including manifesting OTC-deficient heterozygotes) the outcome is better but variable.[28] Most do well with alternative pathway therapy, and the neurodevelopmental outcome is generally good. They too, however, remain at risk for hyperammonemic crises, which carry significant morbidity and mortality.[45]

USE OF PB IN PREGNANCY

Because more young women who are manifesting OTC-deficient heterozygotes or have other partial urea cycle defects are being treated with PB, the question of teratogenicity is raised. In certain ways PB treatment mimics maternal phenylketonuria. Pregnant women with PKU who are not following a low-phenylalanine diet have been found universally to produce children who have microcephaly and mental retardation; and congenital heart defects are also common.[46] The cause is not clear, although animal models suggest that phenylalanine and its metabolites are teratogenic.[47] Because PA is a metabolite of phenylalanine, it could conceivably lead to fetal damage in pregnant women receiving PB; the plasma levels of PA in these women are similar to those in women with maternal PKU.[46] To this point only 1 pregnancy has been reported in the litera-

Table II. Long-term treatment of urea cycle disorders

Disorder*	Citrulline	Arginine free base	Sodium phenylbutyrate†
CPS or OTC deficiency	.170 g/kg/d or 3.8 g/m²/d	—	.450-.600 g/kg/d if <20 kg; 9.9-13.0 g/m²/d in larger patients
Argininosuccinic acid synthetase deficiency	—	.400-.700 g/kg/d or 8.8-15.4 g/m²/d	.450-.600 g/kg/d if <20 kg; 9.9-13.0 g/m²/d in larger patients
Argininosuccinic acid lyase deficiency	—	.400-.700 g/kg/d or 8.8-15.4 g/m²/d	May not be required
Arginase deficiency	—	—	.450-.600 g/kg/d if <20 kg; 9.9-13.0 g/m²/d in larger patients
NAGS deficiency‡	.170 g/kg/d or 3.8 g/m²/d	.170 g/kg/d or 3.8 g/m²/d	.450-.600 g/kg/d if <20 kg; 9.9-13.0 g/m²/d in larger patients

NAGS, N-acetyl glutamate synthase.

*Caloric requirement may be completed with a protein-free formula. In general, the minimum daily protein intake for growth was used: for 1 to 4 months, 1.6 to 1.9 g/kg/d; for 4 to 12 months, 1.7 g/kg/d.; for 1 to 3 years, 1.4 g/kg/d. Daily protein intake may include an essential amino acid formula.

†If intolerant of phenylbutyrate, sodium benzoate + sodium phenylacetate can be given orally at a dose of .250 to .500 g/kg/d each.

‡N-carbamylglutamate may also be given at a dose of .320 to .650 g/kg/d.

ture of a patient receiving PB.[48] This occurred in a woman with partial OTC deficiency who was placed on 280 mg/kg/d phenylbutyrate beginning at 11 weeks' gestation. The plasma ammonia level was not well controlled on this dose, but at 33 weeks a child was well-born and was developing normally when reported at 2 years of age. However, because the PB was not started until the end of the first trimester, this case does not resolve the issue of potential teratogenicity of PB. Recently, 2 additional pregnancies of OTC-deficient women receiving PB have been reported anecdotally to have resulted in well-born infants (James Leonard, personal communications). The actions of PB and PA on gene silencing[49] and cell differentiation[22] support the need for more rigorous evaluation of fetal risk.

OTHER USES OF PA/PB

Serendipity has also influenced the use of PA and PB in other disorders. These compounds have been studied as potential chemotherapeutic agents. PB and PA serve as inhibitors of histone deacetylase and inducers of histone acetylation that inhibit tumor growth; PA works less well as a tumor suppressor than PB.[49]

PB and PA also appear to enhance efficacy of other therapeutic agents including retinoids, interferon-α, suramin, 5-aza-2-deoxycytidine, and hydroxyurea. The enhancement of hydroxyurea makes them potentially valuable in increasing fetal hemoglobin production in hemoglobinopathies.[50] They have also been found to increase cystic fibrosis transmembrane conductance regulator trafficking in vitro by reducing Hsc70 immunoreactivity and could therefore play a role in cystic fibrosis treatment.[51] How any of these other biologic effects of PA and PB may affect individuals with urea cycle disorders is uncertain.

CURRENTLY RECOMMENDED ALTERNATIVE PATHWAY THERAPY

Long-term Therapy

The aim of long-term therapy has been to maintain metabolic control with plasma ammonia concentrations less than twice normal and plasma glutamine levels <1000 µmol/L.[3,42] Table II summarizes the current recommendations for long-term alternative pathway therapy in the different urea cycle disorders. The basic principles are to give adequate nitrogen intake for growth, to provide arginine (or citrulline) as a semiessential amino acid (except in arginase deficiency), and to stimulate alternative pathways of waste nitrogen excretion with PB (400 to 600 mg/kg/d) and arginine (400 to 700 mg/kg/d, in ASS and ASL deficiencies).

Acute Management of Hyperammonemic Crisis

Early identification and treatment of intercurrent hyperammonemic episodes is essential, both because treatment is more effective at lower ammonia levels and because neurologic outcome appears to be a function of duration of severe hyperammonemia and coma.[4] If symptoms have progressed to vomiting and lethargy, plasma ammonia levels are usually >3 to 5 fold normal, and ag-

gressive treatment is warranted. The recommended approach to treating acute hyperammonemia is outlined in Table I. It focuses on ameliorating catabolism and removing nitrogen. Nonprotein calories are provided as glucose (8 to 10 mg/kg/min) with intravenous fat emulsion added to maintain a caloric intake of >80 kcal/kg/d and to minimize endogenous proteolysis. For CPS and OTC deficiencies, intravenous arginine is given as 2 mL/kg of 10% arginine hydrochloride (200 mg/kg, Kabivitrum, Clayton, NC) over a 90-minute period followed by the same dose over a 24-hour period. For ASS and ASL deficiencies, the priming dose of arginine hydrochloride is 6 mL/kg (600 mg/kg) followed by the same dose over a 24-hour period. Sodium bicarbonate should be included in the intravenous fluids to neutralize the acidifying effects of arginine hydrochloride. A priming dose of sodium benzoate/PA (250 mg/kg each; 5.5 g/m^2 for older patients) is administered over a 90-minute period in 25 to 35 mL/kg of 10% glucose (for older patients 400 to 600 mL/m^2 of 10% glucose) followed by the same dose every 24 hours until oral phenylbutyrate therapy can be restarted. Because vomiting often occurs during the priming dose, Zofran (ondansetron hydrochloride), a potent antiemetic, may be given intravenously (0.15 mg/kg) at the start of the infusion. Because 1 g of sodium benzoate contains 160 mg sodium and 1 g of PA contains 147 mg sodium, additional sodium does not need to be provided in the intravenous solution, because hypernatremia can develop. Conversely, hypokalemia can result from urinary potassium losses enhanced by hippurate and PAG excretion, so potassium should be added to the intravenous fluid. If this treatment does not lead to a stabilizing or lowering of ammonia levels within 4 hours, or if the patient's clinical condition worsens, hemodialysis should be started. Alternative pathway therapy should be continued during hemodialysis, because its effects appear to be additive. Notably, hemodialysis decreases plasma concentrations of PAG and hippurate to approximately the same extent as that of urea and creatinine.[15] Monitoring and treatment of increased intracranial pressure are also important.

CONCLUDING REMARKS

Alternative pathway therapy remains a mainstay of both acute and long-term treatment of inborn errors of urea synthesis. Evidence supports continued use at the currently recommended regimen (Tables I and II), with the modification that phenylbutyrate may also be a useful therapy in ASL deficiency. If dialysis is required, alternative pathway therapy should be continued, because its effects are synergistic. Serum drug levels should be monitored during both acute and long-term therapy, especially because a straightforward method is now available. The question of the use of phenylbutyrate during pregnancy remains a difficult one; the risks of fetal damage must be balanced against the risk of hyperammonemia to the mother. Because there are rare individuals who will have side effects from PB sufficient to require its withdrawal, there is still a role for oral sodium benzoate/PA, and it should remain available. Finally, because incorrectly or ambiguously written orders for intravenous sodium benzoate/PA have led to fatal overdoses, it is important to emphasize the importance of checking the calculations for this infrequently used medication.

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REFERENCES

1. Allan JD, Cusworth DC, Dent CE, Wilson VK. A disease, probably hereditary, characterized by severe mental deficiency and a constant gross abnormality of amino acid metabolism. Lancet 1958;1:182-7.
2. Shih VE. Hereditary urea-cycle disorders. In: Grisolia S, Baguena R, Mayor F, editors. The urea cycle. New York: John Wiley; 1976. p. 367.
3. Brusilow SW, Horwich AL. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. 7th ed. New York: McGraw-Hill Information Services Co; 1995. p. 1187-232.
4. Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. N Engl J Med 1984;310:1500-5.
5. Whitington PF, Alonso EM, Boyle JT, Molleston JP, Rosenthal P, Emond JC, et al. Liver transplantation for the treatment of urea cycle disorders. J Inherit Metab Dis 1998;21(Suppl 1):112-8.
6. Lee B, Goss J. Long-term correction of urea cycle disorders. J Pediatr 2001; 138:S62-S71.
7. Morris W. The American Heritage Dictionary. Boston: Houghton Mifflin Co; 1981.
8. Walser M, Batshaw M, Sherwood G, Robinson B, Brusilow S. Nitrogen metabolism in neonatal citrullinaemia. Clin Sci Mol Med 1977;53:173-81.
9. Brusilow SW, Valle DL, Batshaw ML. New pathways of nitrogen excretion in inborn errors of urea synthesis. Lancet 1979;2:452-4.
10. Brusilow SW, Batshaw ML. Arginine therapy of argininosuccinase deficiency. Lancet 1979;1:124-7.
11. Lewis HB. Studies in the synthesis of hippuric acid in the animal organism. J Biol Chem 1914;17:225-31.
12. Sherwin CP, Kennard KS. Toxicity of phenylacetic acid. J Biol Chem 1919; 17:259-64.
13. Brusilow S, Tinker J, Batshaw ML. Amino acid acylation: a mechanism of nitrogen excretion in inborn errors of urea synthesis. Science 1980;207:659-61.
14. Tremblay GC, Qureshi IA. The biochemistry and toxicology of benzoic acid metabolism and its relationship to the elimination of waste nitrogen. Pharmacol Ther 1993;60:63-90.
15. Zimmerman L, Jörnvall H, Bergström J. Phenylacetylglutamine and hippuric acid in uremic and healthy subjects. Nephron 1990;55:265-71.

16. Intravenous sodium phenylacetate/sodium benzoate: clinical investigators' brochure and treatment guidelines. Hunt Valley (MD): Ucyclyd Pharma, Inc; 1996.

17. Green TP, Marchessault RP, Freese DK. Disposition of sodium benzoate in newborn infants with hyperammonemia. J Pediatr 1983;102:785-90.

18. Simell O, Sipila I, Rajantie J, Valle DL, Brusilow SW. Waste nitrogen excretion via amino acid acylation: benzoate and phenylacetate in lysinuric protein intolerance. Pediatr Res 1986; 20:1117-21.

19. Kubota K, Ishizaki T. Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans. Eur J Clin Pharmacol 1991;41:363-8.

20. Oyanagi K, Kuniya Y, Tsuchiyama A, Nakao T, Owada E, Sato J, et al. Nonlinear elimination of benzoate in patients with congenital hyperammonemia. J Pediatr 1987;110:634-6.

21. Mawal Y, Paradis K, Qureshi IA. Developmental profile of mitochondrial glycine N-acyltransferase in human liver. J Pediatr 1997;130: 1003-7.

22. Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor AO, Tompkins AC, et al. A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res 1994;54:1690-4.

23. Brusilow SW, Danney M, Waber LJ, Batshaw M, Burton B, Levitsky L, et al. Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. N Engl J Med 1984;310:1630-4.

24. Piscitelli SC, Thibault A, Figg WD, Tompkins A, Headlee D, Lieberman R, et al. Disposition of phenylbutyrate and its metabolites, phenylacetate and phenylacetylglutamine. J Clin Pharmacol 1995;35:368-73.

25. Brusilow SW. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. Pediatr Res 1991; 29:147-50.

26. Batshaw ML, Brusilow SW, Waber L, Blom W, Brubakk AM, Burton BK, et al. Treatment of inborn errors of urea synthesis: activation of alternative pathways of waste nitrogen synthesis and excretion. N Engl J Med 1982; 306:1387-92.

27. Widhalm K, Koch S, Scheibenreiter S, Knoll E, Colombo JP, Bachmann C, et al. Long-term follow-up of 12 patients with the late-onset variant of argininosuccinic acid lyase deficiency: no impairment of intellectual and psychomotor development during therapy. Pediatrics 1992;89:1182-4.

28. Maestri NE, Brusilow SW, Clissold DB, Bassett SS. Long-term treatment of girls with ornithine transcarbamylase deficiency. N Engl J Med 1996;335:855-9.

29. Griffith AD, Cyr DM, Egan SG, Tremblay GC. Inhibition of pyruvate carboxylase by sequestration of coenzyme A with sodium benzoate. Arch Biochem Biophys 1989;269:201-7.

30. Kalbag SS, Palekar AG. Sodium benzoate inhibits fatty acid oxidation in rat liver: effect on ammonia levels. Biochem Med Metab Biol 1988;40:133-42.

31. Gregus Z, Fekete T, Varga F, Klaassen GC. Availability of glycine and coenzyme A limits glycine conjugation in vivo. Drug Metab Dispos 1992;20: 234-40.

32. LaBrecque DR, Latham PS, Riely CA, Hsia YE, Klatskin G. Heritable urea cycle enzyme deficiency-liver disease in 16 patients. J Pediatr 1979;94:580-7.

33. Anonymous. Sodium phenylbutyrate for urea cycle enzyme deficiencies. Med Lett Drugs Ther 1996;38:105-6.

34. Wiech NL, Clissold DM, MacArthur RB. Safety and efficacy of buphenyl tablets and powder [abstract]. Advances in inherited urea cycle disorders. Satellite to the Seventh International Congress for Inborn Errors of Metabolism, Vienna, 1997.

35. Feillet F, Leonard JV. Alternative pathway therapy for urea cycle disorders. J Inherit Metab Dis 1998;21 (Suppl 1):101-11.

35a. Anadiotis G, Ierardi-Curto L, Kaplan P, Berry GT. Ornithine transcarbamylase deficiency and pancreatitis. J Pediatr 2000 (in press).

36. Praphanphoj V, Boyadjiev SA, Waber LJ, Brusilow SW, Geraghty MT. Three cases of intravenous sodium benzoate and sodium phenylacetate toxicity occurring in the treatment of acute hyperammonaemia. J Inherit Metab Dis 2000;23:129-36.

37. Thibault A, Samid D, Cooper MR, Figg WD, Tompkins AC, Patronas N, et al. Phase I study of phenylacetate administered twice daily to patients with cancer. Cancer 1995;75:2932-8.

38. Gerard JM, Luisiri A. A fatal overdose of arginine hydrochloride. J Toxicol Clin Toxicol 1997;35:621-5.

39. Zimmermann A, Bachmann C, Baumgartner R. Severe liver fibrosis in argininosuccinic aciduria. Arch Pathol Lab Med. 1986;110:136-40.

40. Msall M, Monahan PS, Chapanis N, Batshaw ML. Cognitive development in children with inborn errors of urea synthesis. Acta Paediatr Jpn 1988;30: 435-41.

41. Letarte J, Qureshi IA, Ouellet R, Godar M. Chronic benzoate therapy in a boy with partial ornithine transcarbamylase deficiency. J Pediatr 1985; 106:794-7.

42. Maestri NE, Hauser ER, Bartholomew D, Brusilow SW. Prospective treatment of urea cycle disorders. J Pediatr 1991;119:923-8.

43. Maestri NE, Clissold DB, Brusilow SW. Long-term survival of patients with argininosuccinate synthetase deficiency. J Pediatr 1995;127:929-35.

44. Matsuda I, Nagata N, Matsuura T, Oyanagi K, Tada K, Narisawa K, et al. Retrospective survey of urea cycle disorders: Part l. Clinical and laboratory observations of thirty-two Japanese male patients with ornithine transcarbamylase deficiency. Am J Med Genet 1991;38:85-9.

45. Batshaw ML, Msall M, Beaudet AL, Trojak J. Risk of serious illness in heterozygotes for ornithine transcarbamylase deficiency. J Pediatr 1986; 108:236-41.

46. Scriver CR, Kaufman S, Eisensmith RC, Woo SL. The hyperphenylalaninemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eidtors. The metabolic and molecular bases of inherited disease. 7th ed. New York: McGraw Hill; 1995. p. 1015-75.

47. Denno KM, Sadler TW. Phenylalanine and its metabolites induce embryopathies in mouse embryos in culture. Teratology 1990;42:565-70.

48. Redonnet-Vernhet I, Rouanet F, Pedespan JM, Hocke C, Parrot F. A successful pregnancy in a heterozygote for OTC deficiency treated with sodium phenylbutyrate. Neurology 2000; 54:1008.

49. Lea MA, Randolph VM, Hodge SK.

Induction of histone acetylation and growth regulation in erythroleukemia cells by 4-phenylbutyrate and structural analogs. Anticancer Res 1999;19: 1971-6.

50. Hoppe C, Vichinsky E, Lewis B, Foote D, Styles L. Hydroxyurea and sodium phenylbutyrate therapy in thalassemia intermedia. Am J Hematol 1999;62: 221-7.

51. Rubenstein RC, Egan ME, Zeitlin PL. In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing delta F508-CFTR. J Clin Invest 1997,100:2457-65.

PHARMACOLOGIC MANAGEMENT DISCUSSION

Treatment Recommendations

Several recommendations to ensure optimal use of alternative pathway treatments were discussed and agreed, as follows:

1. Written orders must be checked to avoid overdosage. Used incorrectly, these compounds can be toxic.
2. Alternative pathway treatments should be continued during periods of dialysis.
3. Drug levels should be monitored because of the variability of drug excretion.
4. Continue production of oral phenylacetate and sodium benzoate for individuals who cannot tolerate sodium phenylbutyrate.

Blood Level Determinations

It was considered important to have a test readily available to measure blood levels of Buphenyl (sodium phenylbutyrate), because this is one of the few drugs with a known toxicity profile for which there is currently no routine test available.

Research work on the measurement of drug levels in blood is being carried out by Dr Tuchman's laboratory in Washington, DC, with the goal of creating a system that permits the easy, standardized collection and delivery of samples to the laboratory (on blood spots) for the determination of sodium phenylbutyrate, sodium phenylacetate, and sodium benzoate blood levels. A pilot study is underway to test the method and eventually, it is hoped, to establish a group of laboratories skilled in the measurement of blood levels of these drugs.

Surrogate Markers of Toxicity

Few laboratories offer the measurement of blood levels of the drugs used in the treatment of hyperammonemia. Phenylbutyrate is one of the few drugs with known toxicities for which testing is not routinely available. Efforts are being made to rectify this situation, but in the absence of the ability to measure drug levels in blood, a surrogate marker of toxicity was viewed as better than no marker at all. An anion gap in the serum electrolytes of <15 mEq/L and an anion gap that is rising no more than 6 mEq/L is a good indication that there is no significant accumulation of drug in the blood, and therefore that the development of significant drug toxicity is unlikely.

Dosing Regimens and Drug Stability in Formulas

There was considerable discussion on the optimal dosing frequency for drugs used in the treatment of hyperammonemic crises. The drug regimen recommended for emergency treatment is also discussed in the article by Dr Summar elsewhere in this supplement. For chronic administration of alternative pathway drugs, it was agreed that 3 times per day should be the minimum frequency recommended. The rationale for this was to avoid the toxicities seen with these drugs, which in several instances are related to high peak blood levels, and to link dosage with meals that provide a protein load.

For patients with hyperammonemic symptoms in the early morning, it may be appropriate to give a larger dose late at night, especially if there is no night-time feed. This will prevent the slow accumulation of ammonia during the night, while the patient may be in a catabolic phase.

It is not known how stable the alternative pathway drugs are in the formulas used to feed children with urea cycle disorders. On theoretical grounds alone it is anticipated that the drugs will be stable. Binding of the drugs to components in the formulae, making them less bioavailable, is likely to be more important and Ucyclyd Pharmaceuticals offered to carry out the necessary tests if supplied with a protocol.

Availability of Oral Sodium Benzoate and Phenylacetate

Sodium benzoate and phenylacetate, the first compounds used in the treatment of patients with hyperammonemia, are no longer available as Ucephan, and the patent is currently held by Johns Hopkins Hospital. A change in Food and Drug Administration regulations in 1998 permits any compounding pharmacist to make the drug, but without calling it Ucephan. Currently there are 2 pharmacists compounding sodium benzoate, in North Carolina and California. The group recommended that oral sodium benzoate and oral sodium phenylacetate continue to be made available for individuals who cannot tolerate oral sodium phenylbutyrate.

Drug Toxicity

In children who have had side effects with sodium phenylbutyrate, there is merit in the combined administration of sodium benzoate and sodium phenylbutyrate, because the use of the combination permits lower doses of sodium phenylbutyrate to be administered.

Pregnancy and Teratogenicity of Alternative Pathway Treatments

Little is known about the risk of taking these products when pregnant. It was agreed that it would be helpful to establish and maintain a world-wide

register of pregnant females taking any of these products.

A limited number of pregnancies have occurred while patients were taking sodium phenylbutyrate, but only anecdotal information is available. No known birth defects have been recorded, but a register of such births was viewed as important.

Other Uses of Sodium Phenylacetate and Sodium Phenylbutyrate

Early work on sodium phenylacetate prompted the study of alternative pathway drugs in a variety of diseases including cancer. It is known that depleting glutamate will induce apoptosis in cancer cells in experimental animals. It appears, however, that the doses needed to induce such glutamine depletion in humans would require massive amounts of the drugs. There is ongoing work with these compounds in cystic fibrosis and hemoglobinopathies, but it is too early to know how effective they may be.