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-

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