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Effect of alternative pathway therapy on branched chain amino acid metabolism in urea cycle disorder patients

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Abstract

Urea cycle disorders (UCDs) are a group of inborn errors of hepatic metabolism caused by the loss of enzymatic activities that mediate the transfer of nitrogen from ammonia to urea. These disorders often result in life threatening hyperammonemia and hyperglutaminemia. A combination of sodium phenylbutyrate and sodium phenylacetate/benzoate is used in the clinical management of children with urea cycle defects as a glutamine trap, diverting nitrogen from urea synthesis to alternatives routes of excretion. We have observed that patients treated with these compounds have selective branched chain amino acid (BCAA) deficiency despite adequate dietary protein intake. However, the direct effect of alternative therapy on the steady state levels of plasma branched chain amino acids has not been well characterized. We have measured steady state plasma branched chain and other essential non branched chain amino acids in control subjects, untreated ornithine transcarbamylase deficiency females and treated null activity urea cycle disorder patients in the fed steady state during the course of stable isotope studies. Steady state leucine levels were noted to be significantly lower in treated urea cycle disorder patients when compared to either untreated ornithine transcarbamylase deficiency females or control subjects ($P < 0.0001$). This effect was reproduced in control subjects who had depressed leucine levels when treated with sodium phenylacetate/benzoate ($P < 0.0001$). Our studies suggest that this therapeutic modality has a substantial impact on the metabolism of branched chain amino acids in urea cycle disorder patients. These findings suggest that better titration of protein restriction could be achieved with branched chain amino acid supplementation in patients with UCDs who are on alternative route therapy.

Keywords: Branched chain amino acids; Sodium phenylacetate; Sodium phenylbutyrate; Urea cycle disorders

Introduction

Urea cycle disorders (UCDs) are a group of inborn errors of hepatic metabolism that result in often life-threatening hyperammonemia and hyperglutaminemia [1]. The management of hyperammonemic episodes in these disorders is achieved by dietary protein restriction, supportive management of catabolic stress, and the use of compounds that remove nitrogen by alternative pathways. Alternative pathway therapy includes the use of sodium phenylacetate/benzoate (Ucephan) or sodium phenylbutyrate (Buphenyl) to stimulate the excretion of nitrogen as phenylacetylglutamine and hippuric acid (in the case of Ucephan) [2,3]. Phenylbutyrate does not accumulate in plasma, and within minutes, it is first activated to its CoA ester, and then converted to phenylacetylCoA via β-oxidation [2]. This ultimately is conjugated with glutamine in the liver and kidney to yield phenylacetylglutamine, which is excreted in the urine. Hence, it replaces urea as a means of eliminating excess nitrogen compounds. We have previously shown that the treatment with Ucephan in both control and UCD subjects will increase glutamine flux and decrease total body urea flux directly proportional to the molar conversion of phenylacetate to phenylacetylglutamine [4].

We have observed in our UCD patient population that this therapy leads to a marked fall in serum branched chain amino acids (BCAA) concentrations in spite of apparently adequate levels of total protein intake.

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This observed fall has often preceded a metabolic decompensation. One predicted effect of these compounds might be the dysregulation of global protein synthesis. Phenylbutyrate-induced glutamine depletion in healthy subjects has been shown to exert a profound effect on leucine metabolism including the lowering of plasma leucine concentrations and increasing leucine oxidation [5]. In addition, several conditions leading to hyperammonemia, including liver cirrhosis and idiopathic portal hypertension, a condition characterized by extensive portal-systemic shunting, are invariably associated with a decline in the plasma levels of BCAA [6,7]. This suggests that hyperammonemia and intracellular glutamate depletion may contribute to BCAA deficiency through the stimulation of BCAA transamination. The present study was designed to achieve a deeper understanding of the effect of alternative pathway therapy on branched chain amino acid metabolism in fed UCD patients, asymptomatic untreated ornithine transcarbamylase (OTC)-deficient females, and normal controls.

Materials and methods

The study received prior approval from the Institutional Review Boards for Human Subjects of Baylor College of Medicine. The studies were carried out while the patients were admitted for nitrogen flux studies to assess in vivo urea cycle activity [4]. The use of [^{18}O]urea and [^{15}N]glutamine in these stable isotope studies should not have affected the levels of plasma amino acids based on the low total dose infused [4].

Study subjects

Eleven healthy adult control subjects (six males, ages 19 39 years, and five females, ages 25 42 years) were enrolled. Five subjects with disorders affecting the urea cycle were also enrolled. This group was comprised of two male patients with severe, neonatal-onset ornithine transcarbamylase deficiency (OTCD) and three patients with argininosuccinate synthetase deficiency (ASSD). We also investigated six OTC-deficient females (asymptomatic and untreated). All adult controls were initially studied on a low protein intake [0.4 g/(kg day)]. Five of these subjects were randomly assigned to a group for a second study when in addition to the low protein diet, they received Ucephan treatment [250 mg/(kg day)]. Symptomatic patients with UCD continued on their respective medical regimens in all studies. The untreated asymptomatic OTCD females were studied on the low protein diet [0.4 g/(kg day)]. Two of the symptomatic, treated OTCD females were studied first on low protein intakes and then on low protein intake with Ucephan [250 mg/(kg day)].

Five null UCD patients (three patients with OTCD and two patients with carbamoylphosphate deficiency type I) on sodium phenylbutyrate treatment and two patients with partial OTCD on sodium benzoate were studied retrospectively and their leucine and phenylalanine levels were followed over a period of three months.

Clinical protocol

The clinical protocol was approved by the Baylor College of Medicine Human Subjects Institutional Review Board. The subjects were admitted into the Texas Children's Hospital General Clinical Research Center and were started on the study protocol after physical examination and informed consent. Each subject was started on the assigned level of protein intake and medication (if indicated) for a two-day period of stabilization. Protein intake was monitored by weighing portions before and after each meal. On the third day of the study, after an overnight fast and after a preinfusion blood sampling for the determination of baseline plasma amino acids, the subjects consumed the first of four twice-hourly meals that each supplied $\frac{1}{12}$ of their prior daily protein intake. Blood for plasma amino acids and ammonia was obtained at 0 h preinfusion, and then at 4, 6, and 7.5 h during the infusion of stable isotopes. The levels of branched chain and non-branched chain essential amino acids in the different groups were evaluated in fed individuals at steady state on the third day of the study after a two-day period of stabilization.

Statistical analysis

Three different groups (healthy control subjects, null patients, and asymptomatic OTCD females) were initially compared with a one-way analysis of variance (ANOVA). A P (two-tailed) of less than 0.05 was taken as statistically significant. Within a group, treatment effects (use of phenylbutyrate) were assessed by paired t tests.

Results

Steady state branched chain amino acid levels in UCD subjects in comparison with normal controls and asymptomatic OTCD females

Plasma amino acids were determined in fed control, asymptomatic OTCD, and treated UCD patients after stabilization on the standard low protein diet. Interestingly, steady state serum BCAA (leucine, valine, and isoleucine) levels were found to be significantly

lower ($P < 0.0001$) in null UCD subjects that were treated with sodium phenylbutyrate when compared to normal controls or untreated asymptomatic OTCD females that participated in nitrogen flux studies (Table 1). Other essential non-BCAA (lysine and phenylalanine) were evaluated and no significant differences were found among the three groups while on the same diet (Table 2).

Steady state BCAA levels in control subjects and asymptomatic OTCD females treated and not treated with Ucephan (sodium benzoate/sodium phenylacetate)

Plasma amino acids were determined in a control group after stabilization on a low protein diet and then again after stabilization and treatment with sodium phenylacetate/benzoate. Steady state leucine, valine, and isoleucine levels were significantly diminished in the treatment period as compared to the pre-treatment period ($P < 0.0001$) (Table 3). As expected, steady state glutamine levels were also lower while on treatment ($P < 0.005$). Other essential non-BCAAs were compared and no statistically significant differences were found between the pre-treatment and treatment periods (Table 4). Similarly, branched chain amino acids were evaluated in two OTCD females before and after treatment with phenylacetate/benzoate (Table 5). The three BCAAs were decreased, while no differences were observed among other essential amino acids (Table 6).

Table 1
Steady state serum branched chain amino acids in fed control, asymptomatic OTCD females, and treated UCD patients

Subjects	Plasma concentrations (μmol/L)		
	Leucine	Valine	Isoleucine
Control (N 11)	109 ± 11	173 ± 34	51 ± 13
Asymptomatic OTCD females (N 6)	110 ± 12	180 ± 52	55 ± 14
Treated UCD (N 5)	35 ± 6	87 ± 42	24 ± 14
Significance	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Table 2
Steady state serum non branched chain amino acids in fed controls, asymptomatic OTCD females, and UCD patients

Subjects	Plasma concentrations (μmol/L)				
	Threonine	Lysine	Glutamine	Methionine	Phenylalanine
Control (N 11)	119 ± 31	186 ± 30	518 ± 71	21 ± 5	45 ± 7
Asymptomatic OTCD females (N 6)	126 ± 53	162 ± 29	510 ± 55	24 ± 6	42 ± 3
Treated UCD (N 5)	140 ± 77	163 ± 9	579 ± 100	22 ± 6	38 ± 6
Significance	NS	NS	$P < 0.01$	NS	NS

Table 3
Steady state serum branched chain amino acids and glutamine in control subjects treated with phenylacetate/benzoate

Subjects (N 5)	Plasma concentrations (μmol/L)			
	Leucine	Valine	Isoleucine	Glutamine
Before treatment	106 ± 6	171 ± 33	51 ± 12	495 ± 33
After treatment	40 ± 5	126 ± 33	34 ± 14	438 ± 34
Treatment effect	$P < 0.0001$	$P < 0.001$	$P < 0.001$	$P < 0.005$

Table 4
Steady state serum non branched chain essential amino acids in control subjects treated with phenylacetate

Subjects (N 5)	Plasma concentrations (μmol/L)			
	Methionine	Lysine	Threonine	Phenylalanine
Before treatment	22 ± 3	177 ± 36	128 ± 28	46 ± 7
After treatment	22 ± 4	173 ± 33	134 ± 24	48 ± 4
Treatment effect	NS	NS	NS	NS

Table 5
Effects of sodium phenylacetate on BCAA and glutamine in two asymptomatic OTCD female

Subjects (*N* 2)	Plasma concentrations (μmol/L)			
	Leucine	Isoleucine	Valine	Glutamine
Before treatment	104 ± 9	52 ± 5	199 ± 12	544 ± 40
After treatment	58 ± 13	30 ± 10	129 ± 9	505 ± 63

Table 6
Effects of sodium phenylacetate on non branched chain essential amino acids on two asymptomatic OTCD female

Subjects (*N* 2)	Plasma concentrations (μmol/L)			
	Phenylalanine	Threonine	Methionine	Lysine
Before treatment	40 ± 2	114 ± 6	25 ± 1	218 ± 17
After treatment	40 ± 6	131 ± 3	21 ± 1	192 ± 6

Retrospective study of serum leucine and phenylalanine levels in UCD subjects treated with sodium phenylbutyrate and sodium benzoate

A retrospective study was conducted on five null UCD patients (three OTCD males, two male patients with carbamoylphosphate synthetase deficiency) over a period of three months after initiation of sodium phenylbutyrate therapy. Serum leucine levels declined over a period of three months despite increasing protein supplementation. The patient's protein sufficiency was appropriate as measured by growth and biochemical markers. For example, the lysine and phenylalanine levels remained stable (Figs. 1A and B). These patients also exhibited normal albumin and prealbumin levels (data not shown). Moreover, when leucine levels were evaluated over the same period of time in partial UCD patients who were treated with only sodium benzoate, a similar effect on BCAAs was not observed (Figs. 2A and B).

Discussion

We report that UCD patients treated with either sodium phenylacetate/benzoate or with sodium phenylbutyrate exhibit a selective depression of steady state serum BCAA levels in the face of adequate protein intake as measured by steady state levels of non-branched chain essential amino acids and albumin. In UCD patients, phenylbutyrate is used as a "glutamine trap" causing a decrease in plasma glutamine. This compound decreased plasma glutamine levels even in infants in whom plasma glutamine levels were within normal limits [2]. This effect is achieved via molar conversion of glutamine to phenylacetylglutamine. Glutamine is the most abundant amino acid in the body and comprises two-thirds of the intracellular amino acid pool [8]. Glutamine can be synthesized de novo from glutamate and ammonia in a wide variety of tissues containing glutamine synthetase, and hence, it is considered a non-essential amino acid. However evidence has accumulated that glutamine might also play a role in the regulation of protein homeostasis.

In hypercatabolic dogs adapted to a normocaloric, low protein diet, enteral glutamine supplementation

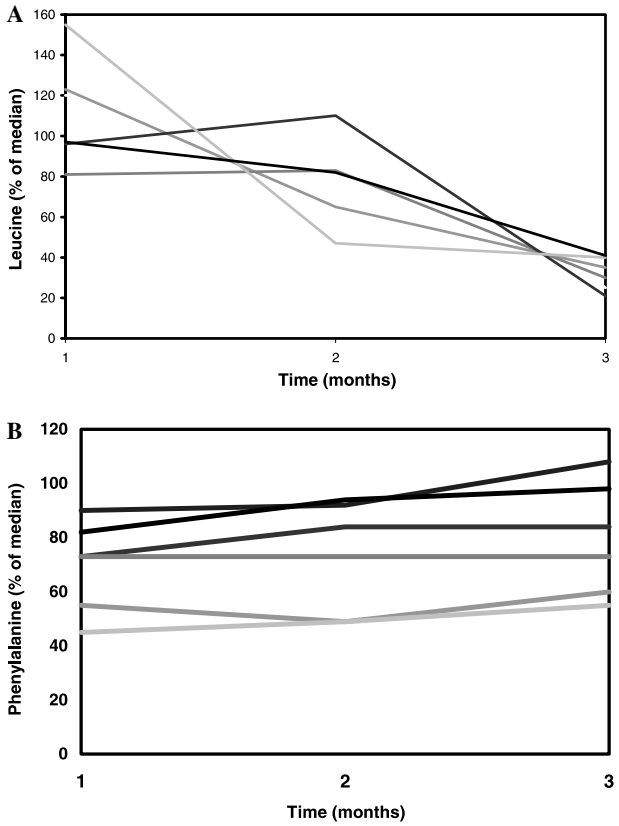

Fig. 1. Retrospective study of serum leucine and phenylalanine levels in five UCD patients treated with sodium phenylbutyrate at one, two, and three months after initiation of treatment. (A) Serum leucine levels. (B) Serum phenylalanine levels.

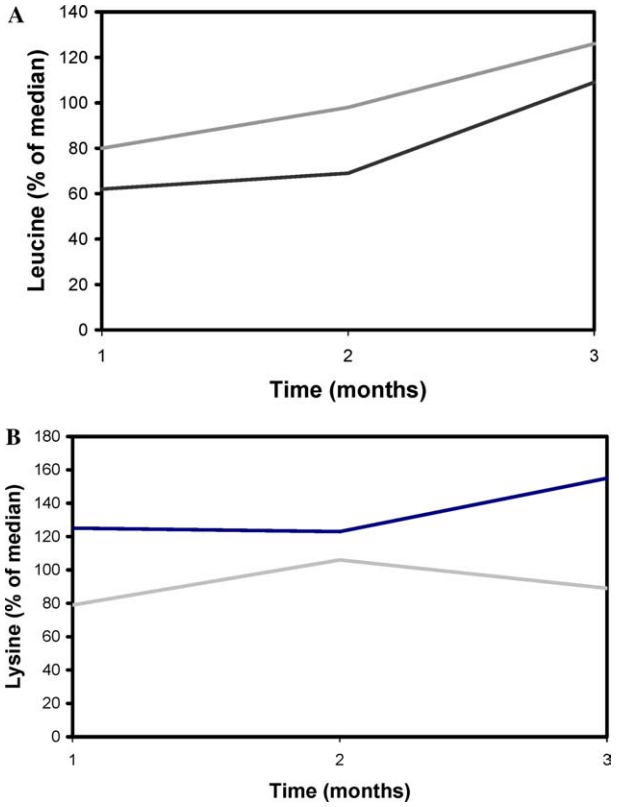

Fig. 2. Retrospective study of serum leucine and phenylalanine levels in two UCD patients treated only with sodium benzoate at one, two, and three months after initiation of treatment. (A) Serum leucine levels. (B) Serum phenylalanine levels.

decreased leucine oxidation. It did so by improving net leucine balance, and thus preserving body protein [9]. This was associated with an approximately 26% reduction in leucine oxidation ($P < 0.05$) with no change in protein release from protein breakdown.

In vitro studies in differentiated Caco-2 enterocyte-like cells demonstrated that inhibition of glutamine synthesis slowed down cell protein synthesis, and a supply of glutamine under these conditions acutely restored protein synthesis in a dose-dependent fashion. This suggests that the maintenance of intracellular glutamine plays a significant physiological role in the control of protein synthesis in a cell line of human origin that exhibits an enterocytic differentiation in vitro [10].

Similar findings in human studies were found when the effect of enteral glutamine on whole body protein metabolism in healthy subjects was studied. Doubling of plasma glutamine concentration obtained through an enteral infusion of L-glutamine in healthy subjects in the postabsorptive state, inhibited leucine oxidation without affecting leucine release from proteolysis. As a consequence, it improved net leucine balance by increasing non-oxidative leucine disposal (NOLD), an index of whole body protein synthesis [11]. Replenishment of muscle glutamine stores has been associated with improved nitrogen balance in conditions of protein wasting [12,13]. It has been shown that a short course treatment with large doses of phenylbutyrate in healthy adults with an intact urea synthetic pathway, could be used to create a model of glutamine depletion [5]. The observed decline in plasma glutamine was associated with decreased estimates of whole body protein synthesis.

In this same study, the effect of phenylbutyrate on leucine kinetics was analyzed. Compared with the control study day, plasma leucine concentrations were lower on the phenylbutyrate treatment day. Leucine oxidation increased in a statistically significant way on the phenylbutyrate day, and non-oxidative leucine disposal decreased. Plasma leucine concentration was at near steady state during each of the isotope infusions. The possibility of any factors affecting the recovery of labeled CO_2 that would affect the measured rates of leucine oxidation was ruled out in this study. Of note is that the observed rise in leucine oxidation occurred at a time when plasma leucine concentration was lowered. Because neither bicarbonate retention nor the ratio of plasma [$^2H_3$]ketoisocaproic (KIC) to [$^2H_3$]leucine enrichment was affected by phenylbutyrate, it was thought that the change in measured rates of leucine oxidation must have resulted from an actual change rather than from a change in the leucine-to-KIC isotopic ratio.

A plausible hypothesis that might explain the cause of BCAA depletion by ammonia scavengers could be based on the excessive formation of phenylacetylglutamine and the subsequent reduction of the intracellular glutamate pool. This pool might be partly restored by intensified BCAA transamination of α-ketoglutarate. To support this hypothesis, a decrease in BCAA levels has been observed in cases of idiopathic portal hypertension, a condition characterized by extensive portal-systemic shunting, and by hyperammonemia and hyperglutaminemia [6,7]. Further support comes from the fact that glutamate production from α-ketoglutarate utilizes BCAA-derived amino groups [14,15] and that hyperammonemia increases the activity of muscle BCAA aminotransferase [16]. It has been shown that the administration of BCAA to rats with hepatic failure has raised the amount of glutamate in brain tissue [17].

Since exogenous hyperammonemia is known to decrease the plasma levels of BCAA, an animal study with ammonium-infused rats was conducted to investigate whether changes in intracellular amino acid concentrations of muscle are associated with that effect [18]. The intracellular amino acid concentrations assessed in this particular study supported the concept under discussion since increased values of glutamine and decreased values of glutamate and alanine were seen with the deficiency of plasma BCAA. The fall in the alanine level could be explained by a decreased availability of glutamate for transamination with pyruvate [19]. The fall in muscle

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