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Minireview

New insights in nutritional management and amino acid supplementation in urea cycle disorders

Fernando Scaglia *

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
Texas Children's Hospital, Houston, TX 77030, USA

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ABSTRACT

Sodium phenylbutyrate is used in the pharmacological treatment of urea cycle disorders to create alternative pathways for nitrogen excretion. The primary metabolite, phenylacetate, conjugates glutamine in the liver and kidney to form phenylacetylglutamine that is readily excreted in the urine. Patients with urea cycle disorders taking sodium phenylbutyrate have a selective reduction in the plasma concentrations of branched chain amino acids despite adequate dietary protein intake. Moreover, this depletion is usually the harbinger of a metabolic crisis. Plasma branched chain amino acids and other essential amino acids were measured in control subjects, untreated ornithine transcarbamylase deficiency females, and treated patients with urea cycle disorders (ornithine transcarbamylase deficiency and argininosuccinate synthetase deficiency) in the absorptive state during the course of stable isotope studies. Branched chain amino acid levels were significantly lower in treated patients with urea cycle disorders when compared to untreated ornithine transcarbamylase deficiency females or control subjects. These results were replicated in control subjects who had low steady-state branched chain amino acid levels when treated with sodium phenylbutyrate. These studies suggested that alternative pathway therapy with sodium phenylbutyrate causes a substantial impact on the metabolism of branched chain amino acids in patients with urea cycle disorders, implying that better titration of protein restriction can be achieved with branched chain amino acid supplementation in these patients who are on alternative pathway therapy.

Introduction

The nutritional management of patients with urea cycle disorders (UCDs) includes restriction of dietary protein along with provision of adequate protein-free energy, essential amino acid supplements, and vitamins and minerals. In addition, the overall treatment involves the prevention or supportive management of catabolic stress, and the stimulation of alternative pathways of nitrogen excretion by the use of pharmacotherapy that promotes the excretion of waste nitrogen. The pharmacological intervention includes the use of sodium phenylacetate/benzoate (ammonul) or sodium phenylbutyrate (buphenyl) to reduce plasma glutamine levels in clinical situations of metabolic decompensation accompanied by hyperammonemia and hyperglutaminemia [1]. Sodium phenylbutyrate is β-oxidized to phenylacetate and the elimination of waste nitrogen is achieved by conjugating glutamine with phenylacetate in the liver and kidney to form phenylacetylglutamine [2]. In the case of Ammonul, the action of sodium phenylacetate is complemented by the addition of sodium benzoate that conjugates glycine to promote the synthesis of hippuric acid. These conjugates are then eliminated in the urine, accounting for a variable fraction of the dose [1,3].

It has been demonstrated that in adults fed a low protein diet, the oral administration of phenylacetate increases glutamine flux and lowers circulating glutamine concentrations [4]. This finding has also been observed in infants with normal plasma glutamine levels by a similar mechanism [1]. However, previously unknown secondary metabolites of phenylbutyrate have been recently identified in urine of normal human subjects and in perfused rat livers [5]. These metabolites are derived from interaction between the metabolism of phenylbutyrate and that of carbohydrates and lipids, and comprise two categories, glucuronides and phenylbutyrate beta-oxidation side products. Low serum levels of branched chain amino acids (BCAA) during the administration of high doses of sodium phenylbutyrate were observed in a study conducted to evaluate long-term survival of patients with argininosuccinate synthetase deficiency [6]. Moreover, we have identified a consistently selective and striking depletion of serum BCAA among patients with UCDs treated with sodium phenylbutyrate despite being on an isocaloric and adequate dietary protein intake. This de-

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* Address: Department of Molecular and Human Genetics, Baylor College of Medicine, Texas Children's Hospital, Clinical Care Center, Suite 1560, 6621 Fannin Street, Mail Code CC1560, Houston, TX 77030, USA. Fax: +1 832 825 4294.
E-mail address: fscaglia@bcm.tmc.edu.

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crease of serum BCAA has often foreshadowed a metabolic crisis accompanied by hyperammonemia and hyperglutaminemia.

One of the potential mechanisms leading to a catabolic state could be caused by the down-regulation of total body protein synthesis due to the BCAA depletion. Phenylbutyrate use in healthy subjects caused glutamine depletion and disrupted BCAA metabolism with increased leucine oxidation [7]. Another study of UCD patients demonstrated that the use of phenylbutyrate had a marked effect on the metabolism of branched chain amino acids [8,9]. In addition, acute depletion of plasma glutamine and BCAA and increased leucine oxidation were observed in prednisone-treated healthy volunteers who received sodium phenylbutyrate for 24 h [10].

The aim of this review is to summarize new observations of amino acid supplementation in UCD patients with a particular focus on the effect of alternative pathway therapy on BCAA metabolism and the function of leucine as a key regulator of protein synthesis.

Study subjects and methods

Subjects

The study was conducted at the Texas Children's Hospital's General Clinical Research Center. The research protocol received prior approval from the Institutional Review Boards for Human Subjects of Baylor College of Medicine. Appropriate informed consent was signed by the study subjects. The study of the role of BCAA in UCD subjects was carried out during admission for investigative studies of nitrogen flux to estimate *in vivo* urea cycle activity [4].

The study subjects who participated included 11 healthy adult control subjects, six asymptomatic and untreated ornithine transcarbamylase deficiency (OTCD) females, and five subjects with UCDs (two severe, neonatal-onset OTCD male patients and three patients with argininosuccinate synthetase deficiency, ASSD). All control subjects were initially evaluated on an isocaloric low natural protein intake [0.4 g/(kg day)] diet for the three-day duration of the study. Five of these control subjects were randomly assigned to also receive sodium phenylbutyrate ($10\ \mathrm{g\ m^{-2}\ d^{-1}}$). The asymptomatic OTCD females were all evaluated on an isocaloric low natural protein intake [0.4 g/(kg day)] diet for the three-day duration of the initial part of the study. Following this, two of these subjects were also given sodium phenylbutyrate ($10\ \mathrm{g\ m^{-2}\ d^{-1}}$) in addition to the same low protein intake. Patients with ASSD (ages 9–13 years) were evaluated on an isocaloric low total protein and amino acid intake [0.4 g/(kg day)] for the three-day duration of the study, with half of their intake from food protein and half derived from essential amino acids. During the three-day duration of the study, the two severe, neonatal-onset males with OTCD were studied on an isocaloric total protein and amino acid intake [1 g/(kg day)] that was an integral part of their usual dietary management, with half of that intake derived from food protein and the other half from essential amino acids.

Five UCD patients with severe neonatal presentation (three OTCD patients and two patients with carbamoylphosphate synthetase I deficiency) who were being treated with sodium phenylbutyrate and two patients with partial OTCD being treated with sodium benzoate were followed retrospectively during a period of three months.

All UCD patients continued with their previously assigned doses of pharmacological therapy throughout the study.

Clinical research protocol

Each study subject was started on the assigned level of protein intake and sodium phenylbutyrate (if indicated) at the time of admission. The estimation of protein intake was done by weighing food portions before and after each meal. On the third day of the study, following an overnight fast and a pre-infusion blood sampling for the analysis of baseline plasma amino acids, the subjects were given four small meals every 2 h. Each meal supplied $^{1}/_{12}$ of the prior daily protein intake. A blood sample for plasma amino acids and ammonia was obtained at baseline (0 h), and at 4, 6, and 7.5 h during the infusion of the stable isotopes (sampling was done 1.5–2 h after the intake of the small meals). Serum BCAA and other essential non-BCAA in these diverse groups were measured at the plateau phase of isotopic enrichments in the absorptive state during the course of the studies [8].

Results

All serum BCAA levels were significantly lower in severely deficient UCD patients treated with sodium phenylbutyrate when compared to normal subjects or untreated asymptomatic OTCD females. No significant differences were found in other essential non-BCAAs among the three groups [8]. Fairly similar results were encountered when plasma amino acids were measured in a group of control subjects and in two asymptomatic OTCD females after stabilization on their assigned diet, compared with the same two groups while taking phenylbutyrate treatment and receiving the same low protein intake [0.4 g/(kg day)]. Furthermore, a retrospective study in which serum leucine levels were followed in UCD subjects treated with either sodium phenylbutyrate or sodium benzoate demonstrated a decrease of serum leucine levels in the group receiving sodium phenylbutyrate. However, a similar effect was not observed in the group treated with sodium benzoate. The caloric intake and protein sufficiency in all of these subjects was adequate by evaluation of their growth, diet records, and biochemical markers including albumin and prealbumin levels [8].

Discussion

Glutamine as a positive regulator of protein biosynthesis

Glutamine comprises 60% of the free amino acids in muscle and is the most abundant amino acid in the body [11]. It is a non-essential amino acid since it is synthesized *de novo* from glutamate and ammonia by the cytosolic enzyme glutamine synthetase [12]. Although a non-essential amino acid, it plays an important role in protein homeostasis. Furthermore, since the capacity to produce appropriate amounts of glutamine can be overwhelmed by catabolic stress associated with illness, this amino acid may become "conditionally essential" as the observed protein wasting will be reflected on the depletion of skeletal muscle glutamine [12]. Glutamine exerts a pivotal role in regulating protein homeostasis and inhibiting protein degradation [13]. It has been shown that replenishment of glutamine leads to an improvement of nitrogen balance in periods of metabolic stress associated with protein catabolism [14]. Glutamine administered enterally limits amino acid oxidation (including leucine oxidation) and has a sparing effect on whole-body amino acids in hypercatabolic dogs during the fed state [15]. Two different ways of supplementing clinical nutrition products with glutamine, either with free glutamine or with a glutamine-rich protein source, were evaluated in hypercatabolic rats treated with glucocorticoids. Glutamine provided as dietary protein was extensively metabolized by the splanchnic tissues and did not influence peripheral glutamine status to the same extent as glutamine provided as the free amino acid [16]. Glutamine depletion induced by inhibition of glutamine synthetase also led to a marked reduction of protein synthesis in cultured human enterocytes [17]. Regardless of the route of supply, glutamine