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Sodium phenylbutyrate decreases plasma branched-chain amino acids in patients with urea cycle disorders

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ABSTRACT

Sodium phenylbutyrate (NaPBA) is a commonly used medication for the treatment of patients with urea cycle disorders (UCDs). Previous reports involving small numbers of patients with UCDs have shown that NaPBA treatment can result in lower plasma levels of the branched-chain amino acids (BCAA) but this has not been studied systematically. From a large cohort of patients (n = 553) with UCDs enrolled in the Longitudinal Study of Urea Cycle Disorders, a collaborative multicenter study of the Urea Cycle Disorders Consortium, we evaluated whether treatment with NaPBA leads to a decrease in plasma BCAA levels. Our analysis shows that NaPBA use independently affects the plasma BCAA levels even after accounting for multiple confounding covariates. Moreover, NaPBA use increases the risk for BCAA deficiency. This effect of NaPBA seems specific to plasma BCAA levels, as levels of other essential amino acids are not altered by its use. Our study, in an unselected population of UCD subjects, is the largest to analyze the effects of NaPBA on BCAA metabolism and potentially has significant clinical implications. Our results indicate that plasma BCAA levels should to be monitored in patients treated with NaPBA since patients taking the medication are at increased risk for BCAA deficiency. On a broader scale, these findings could open avenues to explore NaPBA as a therapy in maple syrup urine disease and other common complex disorders with dysregulation of BCAA metabolism.

1. Introduction

Sodium phenylbutyrate (NaPBA) is a nitrogen-scavenging agent that is used in the routine management of patients with urea cycle disorders (UCDs) [1–4]. Phenylacetate, the active metabolite generated from NaPBA, conjugates glutamine to form phenylacetylglutamine which is excreted in the urine. Thus, NaPBA provides an alternative route for the disposal of waste nitrogen by diverting waste nitrogen from entering the urea cycle and is used to prevent hyperammonemia. However, NaPBA has diverse biological effects including histone deacetylase inhibition, reduction of endoplasmic reticular stress, modulation of protein phosphorylation, inhibition of adipogenesis, and improvement of glucose homeostasis [5–7]. NaPBA also has been noted to affect the metabolism of the branched-chain amino acids (BCAA) [8].

Low levels of plasma BCAA in patients with UCDs were first noted in a long-term follow-up of 24 patients with citrullinemia who participated in the early studies of the nitrogen-scavenging agents (NaPBA, sodium phenylacetate, and sodium benzoate) [9]. Small studies in control subjects and patients with UCDs have since demonstrated that low plasma BCAA levels are associated with use of NaPBA [8,10,11]. In contrast, decreased BCAA levels have not been observed with sodium benzoate [11]. An initial survey of plasma BCAA levels in patients with UCDs (n = 183) enrolled in the Longitudinal Study of Urea Cycle Disorders, a natural history study conducted by the Urea Cycle Disorders Consortium, demonstrated that patients administered NaPBA had lower BCAA levels relative to those who were not on the medication [12,13]. However, patients with disorders that result in a proximal blockade in ureagenesis, those with frequent metabolic decompensations, and those with more severe protein restriction, are more likely to be prescribed NaPBA. Detailed analyses accounting for covariates that could

Abbreviations: NaPBA, sodium phenylbutyrate; UCD, urea cycle disorder; BCAA, branched-chain amino acids; RDCRN, Rare Diseases Clinical Research Network; GLM, generalized linear model; OTCD, ornithine transcarbamylase deficiency; BCKDK, branched-chain ketoacid dehydrogenase kinase; BCKDC, branched-chain ketoacid dehydrogenase complex; MSUD, maple syrup urine disease.

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confound these observations have not been conducted. In the present study, we evaluated a large population of patients with a variety of UCDs to test whether NaPBA use was independently associated with low BCAA levels even when accounting for potential confounders. In addition, we assessed whether the dose of NaPBA correlates with BCAA levels and whether low BCAA levels increase the risk of hyperammonemia as correlations between these factors, if they exist, could have implications on the management of patients with UCDs.

2. Materials and methods

2.1. Patient population and data collection

Data were collected as part of the Longitudinal Study of Urea Cycle Disorders (ClinicalTrials.gov NCT00237315) [12]. This is a natural history study conducted by the Rare Diseases Clinical Research Network's (RDCRN) Urea Cycle Disorders Consortium (UCDC) that includes 14 academic center sites in the United States, Canada, and Europe [12]. Data from all centers were collected in a standardized format as detailed in the manual of operations and were entered into the electronic database maintained by the Data Management and Coordinating Center of the RDCRN. The data obtained from the enrollment visit were used in the analyses.

We collected data from 611 subjects. Fifty-eight subjects were excluded from the analysis because BCAA levels were not available at the enrollment visit or because of data entry errors. Fifteen subjects who were taking BCAA supplements at enrollment were also included in the primary analysis. For each of the remaining 553 subjects, the following data were collected from the initial enrollment visit: UCD diagnosis, onset of disease (neonatal vs. later onset), age at enrollment, gender, daily reported protein intake (g/kg/day), NaPBA use, and plasma levels of BCAA, other essential amino acids, albumin, and prealbumin. Plasma samples for amino acid analyses were collected after a 3-hour fast and before administration of the nitrogen-scavenging medication. Dietary data were collected and analyzed based on a 3-day diet; when such data were not available, a 24-hour recall was utilized. The laboratory assessments were performed at the local CLIA-certified laboratories. For the analysis of BCAA deficiency, the following thresholds for normal range were used: <30 μMol/L for leucine, <10 μMol/L for isoleucine, and <70 μMol/L for valine.

2.2. Hyperammonemia analysis

Symptomatic hyperammonemia episodes were defined in the manual of operations and recorded when plasma ammonia was greater than 100 μM and required an ER visit, hospitalization, or an unscheduled clinic visit. Ammonia levels were measured at the local facilities where patients presented for evaluation of hyperammonemia. The number of hyperammonemic episodes was recorded based on patient report which was confirmed by review of medical records whenever possible. To analyze whether low plasma BCAA levels conferred a higher risk for hyperammonemia, we calculated the odds ratio of a hyperammonemic event occurring within 12 months of enrollment in subjects with plasma levels of at least two of the three BCAA in the lowest quartile vs. those with at least two of the three BCAA in the highest quartile.

2.3. Statistical analysis

Two-sample comparisons were performed using Mann–Whitney U test. Chi-square analysis was used for comparison of proportions. These statistical analyses and calculations of odds ratios with confidence intervals were performed using GraphPad Prism v 6.03.

To account for the covariates that influence BCAA and other essential amino acid levels, we performed generalized linear model (GLM) analysis. For each amino acid, data was available for all other covariates in 333 study subjects and these subjects were included in subsequent GLM analysis. The plasma levels of each BCAA was the dependent variable while the continuous variables — age and daily protein intake, and categorical variables — gender, type of UCD, onset of presentation, NaPBA use, plasma albumin and prealbumin levels were independent variables. The laboratory tests for patients enrolled in the Longitudinal Study of Urea Cycle Disorders are analyzed at different laboratories. Thus, we used standard estimates of normal ranges for the GLM analysis. Albumin was converted into a categorical variable (normal versus abnormal) based on the following normal values for age (0–30 days, 2.9–5.5 g/dL; 1–3 months g/dL, 2.8–5.0 g/dL; 4–11 months, 3.9–5.1 g/dL and ≥1 year, 3.7–5.5 g/dL). Prealbumin was converted to a categorical variable (normal vs. abnormal) based on the following: 0–6 days, 4–20 mg/dL; 7–41 days, 8–25 mg/dL; ≥42 days, 18–44 mg/dL. The analysis was completed using the GLM function in R project for Statistical computing (http://www.R-project.org/) [14]. Leucine, isoleucine, valine and other essential amino acids did not fit a normal distribution, thus these variables were fit to a gamma distribution. In GLM, the analysis was performed with the gamma family and inverse link function. The complete model was compared to the null model. The models are listed as follows:

Null model:

$$\begin{aligned}\text{Plasma leucine} = \mu &+ A1(\text{age}) + A2(\text{gender}) + A3(\text{onset}) \\ &+ A4(\text{UCD diagnosis}) + A5(\text{protein intake}) \\ &+ A6(\text{plasma prealbumin}) + A7(\text{plasma albumin})\end{aligned}$$

Full model:

$$\begin{aligned}\text{Plasma leucine} = \mu &+ A1(\text{age}) + A2(\text{gender}) + A3(\text{onset}) \\ &+ A4(\text{UCD diagnosis}) + A5(\text{protein intake}) \\ &+ A6(\text{plasma prealbumin}) + A7(\text{plasma albumin}) \\ &+ A8(\text{NaPBA use}).\end{aligned}$$

This analysis was repeated for isoleucine, valine, and other essential amino acids. Significance of the coefficients was calculated in R. Significance of the model comparison was calculated using the difference of the −2(ln_likelihood), and this was compared to a chi-square distribution with one degree of freedom (given 9 degrees of freedom in the full model versus 8 degrees of freedom in the null model). Interaction between significant independent variables (coefficient $p < 0.05$) was examined by comparing the full model to the full model with the addition of an interaction term. GLM was also used to examine the effect of NaPBA dose on BCAA levels. For subjects weighing less than 20 kg ($n = 83$), NaPBA dose was expressed in mg/kg. The participants were further subdivided by dose into three groups [<250 mg/kg ($n = 30$), 250–500 mg/kg ($n = 39$), and >500 mg/kg ($n = 14$)]. For subjects weighing more than 20 kg ($n = 90$), NaPBA dose was expressed in g/m^2 body surface area. Analogous to the participants weighing less than 20 kg, we subdivided participants into three groups [NaPBA dose <5 g/m^2 ($n = 16$), 5–10 g/m^2 ($n = 44$), and >10 g/m^2 ($n = 30$)]. Separately, for subjects weighing less than 20 kg or more than 20 kg, the null model (as above) was compared to a model that included the independent variables in the null model and an additional independent variable for dosage group. In this study, 39 statistical tests were performed, and we corrected for multiple comparisons in order to reduce type 1 error. We used the conservative Bonferroni correction and at an α of 0.05 an uncorrected p-value of 0.00128 was treated as significant.

3. Results

3.1. Study population

The study population is summarized in Table 1. Of the 553 subjects, 212 (38%) were on NaPBA and 341 (62%) were not taking the