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Glutamine and hyperammonemic crises in patients with urea cycle disorders☆,☆☆

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ABSTRACT

Blood ammonia and glutamine levels are used as biomarkers of control in patients with urea cycle disorders (UCDs). This study was undertaken to evaluate glutamine variability and utility as a predictor of hyperammonemic crises (HACs) in UCD patients.

Methods: The relationships between glutamine and ammonia levels and the incidence and timing of HACs were evaluated in over 100 adult and pediatric UCD patients who participated in clinical trials of glycerol phenylbutyrate.

Results: The median (range) intra-subject 24-hour coefficient of variation for glutamine was 15% (8–29%) as compared with 56% (28%–154%) for ammonia, and the correlation coefficient between glutamine and concurrent ammonia levels varied from 0.17 to 0.29. Patients with baseline (fasting) glutamine values >900 μmol/L had higher baseline ammonia levels (mean [SD]: 39.6 [26.2] μmol/L) than patients with baseline glutamine ≤900 μmol/L (26.6 [18.0] μmol/L). Glutamine values >900 μmol/L during the study were associated with an approximately 2-fold higher HAC risk (odds ratio [OR] = 1.98; p = 0.173). However, glutamine lost predictive significance (OR = 1.47; p = 0.439) when concomitant ammonia was taken into account, whereas the predictive value of baseline ammonia ≥1.0 upper limit of normal (ULN) was highly statistically significant (OR = 4.96; p = 0.013). There was no significant effect of glutamine >900 μmol/L on time to first HAC crisis (hazard ratio [HR] = 1.14; p = 0.813), but there was a significant effect of baseline ammonia ≥1.0 ULN (HR = 4.62; p = 0.0011).

Conclusions: The findings in this UCD population suggest that glutamine is a weaker predictor of HACs than ammonia and that the utility of the predictive value of glutamine will need to take into account concurrent ammonia levels.

Abbreviations: GPB, glycerol phenylbutyrate (generic name for glyceryl tri (4-phenylbutyrate), also referred to as HPN-100 or RAVICTI®); NaPBA, sodium phenylbutyrate; UCD, urea cycle disorder.

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1. Introduction

Urea cycle disorders (UCDs) are inborn errors of metabolism involving deficiencies of enzymes or transporters involved in the conversion of ammonia to urea, which result in the accumulation of toxic levels of ammonia in affected patients. Medical management of UCDs is aimed at reducing ammonia levels to within normal limits through the restriction of protein intake and the use of alternate pathway drugs to enhance waste nitrogen excretion.

Blood ammonia and glutamine levels are widely used as biomarkers of disease control in UCD patients. However, blood ammonia levels exhibit considerable daily variability, even among comparatively stable and well-controlled UCD patients [1], and can be affected by blood collection techniques. Plasma glutamine is less affected than ammonia by blood sampling procedures, but glutamine levels also vary over 24 h, reportedly being highest after fasting [2–7]. Fasting ammonia levels have been shown to correlate strongly with total daily ammonia exposure and to be a strong predictor of hyperammonemic crises (HACs) [1]. Glutamine levels exceeding 900 or 1000 μmol/L are commonly taken as indicative of inadequate disease control and a harbinger of HACs [2–6]. However, a recent study by Lee et al. suggested that glutamine appears a weaker predictor of HACs than ammonia [1].

The objective of this study was to extend the work of Lee et al. [1] to compare the 24-h variability of glutamine and ammonia, and to evaluate the utility of glutamine compared with ammonia as an independent predictor of HACs.

2. Methods

2.1. Clinical trials

We performed a post-hoc pooled analysis of data from clinical trials of glycerol phenylbutyrate (GPB, HPN-100, RAVICTI®; Horizon Therapeutics, Brisbane, CA) in pediatric and adult UCD patients. The clinical trials have been described in detail elsewhere [8–11]. Blood samples for 24-hour ammonia and glutamine levels were collected during steady-state dosing with GPB or sodium phenylbutyrate (NaPBA) in a Phase 2, open-label, crossover study in 10 adult UCD patients [11]. Blood samples for evaluating the comparative utility of glutamine vs. ammonia in predicting HACs were collected from 100 stable adult and pediatric UCD patients during GPB dosing in one of three 12-month safety extension studies [8–10].

All study protocols and informed consents were reviewed and approved by the Investigational Review Board of each participating institution prior to study initiation. Informed consent was obtained from all patients prior to being included in the study. For all studies, eligible patients had a confirmed or clinically suspected UCD and had been receiving NaPBA prior to enrollment. Major exclusion criteria included liver transplant, hypersensitivity to PBA, and laboratory abnormalities or ECG findings viewed as clinically significant by the Investigator. In all studies, patients received GPB three times daily (or more frequently in small children to match their prior NaPBA schedule) at a daily dose equivalent to their previously prescribed NaPBA dose.

2.2. Ammonia and glutamine measurements

During the crossover study, serial venous blood samples for ammonia and glutamine analyses were collected over 24 h after the patient had received 1 to 2 weeks of steady-state dosing with either NaPBA or GPB. During the 12-month studies, fasting blood samples for ammonia and glutamine analyses were collected monthly or quarterly and information on HACs was recorded. Baseline values were defined as the screening or month 0 value when the patient was on NaPBA prior to receiving GPB. An HAC was defined as compatible clinical symptoms associated with one or more ammonia levels ≥100 μmol/L. Ammonia and HAC data were also collected retrospectively for up to 12 months prior to enrollment in the GPB studies while patients were receiving NaPBA. Ammonia and glutamine concentrations were measured by an accredited hospital laboratory at each study site and ammonia values were normalized to a standard range of 9 to 35 μmol/L.

2.3. Statistical methods

Glutamine and ammonia levels over 24 h following dosing with GPB or NaPBA in the crossover study were summarized using descriptive statistics to compare overall and intra-subject variability in glutamine as compared with ammonia. We summarized baseline subject characteristics including HAC frequency, baseline glutamine and ammonia levels and demographics for patients enrolled in the 12-month studies.

Correlations between baseline ammonia and glutamine levels in the 12-month studies were calculated using the Spearman's rank correlation method, which is robust to outliers and doesn't assume normality of data. The percentage of patients experiencing an HAC was determined based on categorical glutamine levels at baseline (≤900 μmol/L vs >900 μmol/L) and baseline ammonia levels (<0.5, 0.5–0.99, and ≥1.0 times the upper limit of normal [ULN]) and evaluated by a test of association based on a Fisher's exact test. We evaluated the longitudinal effect of varying glutamine over time on the odds of having an HAC event within the next 3 months through logistic general estimating equation (GEE) regression models [12] using the same glutamine categories and separate models using continuous glutamine (with beta regression transformations to represent odds ratios in terms of increases of 100 or 200 μmol/L in glutamine). The same regression models were repeated with the inclusion of baseline ammonia categories (<0.5, 0.5–0.99, and ≥1.0 ULN) and patient demographic criteria (categorical age, gender, race) to evaluate changes in the association between glutamine and HAC events controlling for ammonia and patient demographics. Similar analyses were performed including only patients with baseline ammonia >0.5 ULN. Lastly, the time to first HAC was evaluated through Kaplan–Meier analyses and Cox proportional hazards regression models with independent predictors of glutamine and ammonia categories similar to above.

Table 1
Baseline characteristics for patients enrolled in 12-month studies (N = 100)[a].

Gender: (%)	
Male	33.0
Female	67.0
Race: (%)	
White	81.0
Nonwhite	19.0
Age (years): mean (SD)	19.6 (15.9)
Age group: (%)	
Pediatric: <18 years	49.0
Adult: ≥18 years	51.0
Baseline glutamine (μmol/L): (n = 96)	
Mean (SD)	740.1 (234.6)
Median (25th, 75th percentiles)	704.5 (586.5, 829.5)
Patients with baseline glutamine >900 μmol/L: %	18.0
Baseline ammonia (μmol/L)	
Mean (SD)	28.8 (19.9)
Median (25th, 75th percentiles)	28.9 (11.0, 37.5)
Patients with baseline ammonia: %	
0–0.49 ULN	39.0
0.5–0.99 ULN	34.0
≥1.0 ULN	27.0
Number of HACs	
Pre-study	54
During study	27
Patients with ≥1 HAC: %	
Pre-study	30.0
During study	19.0

[a] Baseline values for ammonia and glutamine represent the values at the time of enrollment into the glycerol phenylbutyrate clinical trials, at which time patients had been taking sodium phenylbutyrate for months to years. HAC: hyperammonemic crisis; ULN: upper limit of normal.

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