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Urinary phenylacetylglutamine as dosing biomarker for patients with urea cycle disorders

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ABSTRACT

We have analyzed pharmacokinetic data for glycerol phenylbutyrate (also GT4P or HPN-100) and sodium phenylbutyrate with respect to possible dosing biomarkers in patients with urea cycle disorders (UCD).

Study design: These analyses are based on over 3000 urine and plasma data points from 54 adult and 11 pediatric UCD patients (ages 6–17) who participated in three clinical studies comparing ammonia control and pharmacokinetics during steady state treatment with glycerol phenylbutyrate or sodium phenylbutyrate. All patients received phenylbutyric acid equivalent doses of glycerol phenylbutyrate or sodium phenylbutyrate in a cross over fashion and underwent 24-hour blood samples and urine sampling for phenylbutyric acid, phenylacetic acid and phenylacetylglutamine.

Results: Patients received phenylbutyric acid equivalent doses of glycerol phenylbutyrate ranging from 1.5 to 31.8 g/day and of sodium phenylbutyrate ranging from 1.3 to 31.7 g/day. Plasma metabolite levels varied widely, with average fluctuation indices ranging from 1979% to 5690% for phenylbutyric acid, 843% to 3931% for phenylacetic acid, and 881% to 1434% for phenylacetylglutamine. Mean percent recovery of phenylbutyric acid as urinary phenylacetylglutamine was 66.4 and 69.0 for pediatric patients and 68.7 and 71.4 for adult patients on glycerol phenylbutyrate and sodium phenylbutyrate, respectively. The correlation with dose was strongest

Abbreviations: ASL, argininosuccinate lyase deficiency; ASS, argininosuccinate synthetase deficiency; AUC_{0-24}, 24 hour area under the curve; CV%, coefficient of variation; DSMB, Data Safety and Monitoring Board; GPB, glycerol phenylbutyrate (generic name for glyceryl tri (4-phenylbutyrate), also referred to as HPN-100); ITT, intention to treat; NaPBA, sodium phenylbutyrate; $NH3_{24\text{-hour AUC}}$, ammonia 24-hour area under the curve; OTC, ornithine transcarbamylase deficiency; PAA, phenylacetic acid; PAGN, phenylacetylglutamine; PBA, phenylbutyric acid; PK, pharmacokinetic; UCD, urea cycle disorder; ULN, upper limit of normal; $U\text{-}PAGN_{24\text{-hour Excr}}$, PAGN excreted in urine over 24 h.

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for urinary phenylacetylglutamine excretion, either as morning spot urine ($r = 0.730$, $p < 0.001$) or as total 24-hour excretion ($r = 0.791$ $p < 0.001$), followed by plasma phenylacetylglutamine $AUC_{24\text{-hour}}$, plasma phenylacetic acid $AUC_{24\text{-hour}}$ and phenylbutyric acid $AUC_{24\text{-hour}}$. Plasma phenylacetic acid levels in adult and pediatric patients did not show a consistent relationship with either urinary phenylacetylglutamine or ammonia control.

Conclusion: The findings are collectively consistent with substantial yet variable pre-systemic (1st pass) conversion of phenylbutyric acid to phenylacetic acid and/or phenylacetylglutamine. The variability of blood metabolite levels during the day, their weaker correlation with dose, the need for multiple blood samples to capture trough and peak, and the inconsistency between phenylacetic acid and urinary phenylacetylglutamine as a marker of waste nitrogen scavenging limit the utility of plasma levels for therapeutic monitoring. By contrast, 24-hour urinary phenylacetylglutamine and morning spot urine phenylacetylglutamine correlate strongly with dose and appear to be clinically useful non-invasive biomarkers for compliance and therapeutic monitoring.

1. Introduction

Urea Cycle Disorders (UCDs), which include several inherited enzyme and transporter deficiencies, result in the accumulation of toxic levels of ammonia in the blood and brain and can present in the neonatal period or later in life depending on the severity and type of defect [1–3]. Control of hyperammonemia, the major cause of morbidity and mortality in UCD patients, is a major objective of treatment [4,5].

UCD patients whose symptoms are not adequately controlled with diet alone are generally treated with alternate pathway drugs such as sodium phenylbutyrate, which is approved in the US (trade name: BUPHENYL®) (sodium phenylbutyrate) (Powder and Tablets) and Europe (trade name: AMMONAPS®) for the chronic treatment of UCDs and lowers ammonia by enhancing excretion of waste nitrogen in the form of phenylacetylglutamine. Although sodium phenylbutyrate has been used for the treatment of UCDs since at least 1979, comparatively little information is available to guide physicians regarding its optimal dosing [6–8].

Blood ammonia is routinely evaluated; however, ammonia values vary up to 10-fold over the course of a day, even in well controlled patients, a fact which limits the use of random blood ammonia for dose adjustment [9,10]. The major metabolites of sodium phenylbutyrate and glycerol phenylbutyrate metabolites, phenylbutyric acid, phenylacetic acid and phenylacetylglutamine, all have comparatively short circulating half lives and vary many-fold during the day [9,10]. For example, in a study of pediatric UCD patients with three times daily dosing of sodium phenylbutyrate or glycerol phenylbutyrate, daily plasma phenylacetic acid values ranged from <1 to 148 μg/mL on sodium phenylbutyrate and <1 to 244 μg/mL on glycerol phenylbutyrate [10].

Moreover, in adult UCD patients, Lee et al. have reported differences in systemic exposure to PBA (plasma PBA $AUC_{0-24\ h}$ of 740 vs 540 μg·h/mL) despite nearly identical urinary recovery of the administered dose of PBA as PAGN (54%) after sodium phenylbutyrate and glycerol phenylbutyrate treatment [9]. A similar disparity between systemic exposure and urinary recovery of administered dose of PBA as sodium phenylbutyrate and glycerol phenylbutyrate has also been reported in pediatric UCD patients [10]. These findings suggest that metabolite blood levels may not fully reflect waste nitrogen removal and exhibit variability which limits their utility for therapeutic monitoring.

Glycerol phenylbutyrate is an investigational agent being developed for UCDs. It has the same mechanism of action as sodium phenylbutyrate, except that, unlike sodium phenylbutyrate which is a salt, glycerol phenylbutyrate is a short chain triglyceride consisting of 3 molecules of phenylbutyric acid attached to glycerol in ester linkage that contains no sodium and is hydrolyzed in the small intestine by pancreatic lipases to release phenylbutyric acid [8,9,11]. Upon absorption, phenylbutyric acid is converted via β-oxidation to phenylacetic acid, which is conjugated with L-glutamine by phenylacetyl CoA:L glutamine N acetyltransferase found in primates liver and kidney to form PAGN or phenylacetylglutamine, which is excreted in the urine and mediates excretion of waste nitrogen [13,14]. The glycerol phenylbutyrate clinical trials, for which sodium phenylbutyrate has served as the approved comparator, have afforded the opportunity to systematically evaluate the clinical utility of blood and urine metabolites as dosing biomarkers.

2. Materials and methods

2.1. Study design and treatments

2.1.1. Clinical studies

Pharmacokinetic data from 3 switch over studies in UCD patients are presented in these analyses. Patients received exclusively sodium phenylbutyrate or the equivalent dose of glycerol phenylbutyrate during each period. Studies UP1204-003 and HPN-100-005 were open-label, fixed sequence switchover studies completed by 10 adult and 11 pediatric (6–17 yr) UCD patients, respectively. Study HPN-100-006 was a pivotal, randomized, active-controlled, cross-over study completed by 44 adult UCD patients and designed to establish the non-inferiority of glycerol phenylbutyrate to sodium phenylbutyrate as assessed by venous ammonia. In all three studies, patients were on a stable dose of sodium phenylbutyrate and were clinically controlled at entry. Patients received sodium phenylbutyrate or an equivalent dose of glycerol phenylbutyrate divided into 3 daily doses taken with meals for 7–14 days, sufficient to reach steady state (9. 10, 11, 12). At the end of each period, in a controlled clinical setting, serial blood samples (8 to 11 samples) for measurement of ammonia and drug metabolites were obtained as well as urine samples for measurement of phenylbutyric acid, phenylacetic acid and phenylacetylglutamine. The similarity of study design and measurements allows pooling of the data across all three studies.

2.1.2. Biochemical analyses

Sodium phenylbutyrate and glycerol phenylbutyrate metabolites including phenylbutyric acid, phenylacetic acid, and phenylacetylglutamine were measured by a validated liquid chromatography tandem mass spectrometry method whereby data were acquired and processed (integrated) using Analyst (version 1.4) (Applied Biosystems, Inc.) and the peak area information analyzed in relation to separate standard curves for phenylbutyric acid, phenylacetic acid, and phenylacetylglutamine at the bioanalytical laboratory, Quest Pharma Services [9,10,12,15]. Although other metabolites of phenylbutyric acid such as phenylbutyrlglutamine or phenylacetylglycine have been reported in humans [16], these metabolites were not detected in plasma samples and accounted for less than 1% of the administered doses of phenylbutyric acid in adult UCD patients [9] and, therefore, were not evaluated in the present study. Venous ammonia was measured by the accredited hospital laboratory at each site.

2.1.3. Pharmacokinetic and ammonia sampling

Blood samples for analysis of venous ammonia, phenylbutyric acid, phenylacetic acid and phenylacetylglutamine (major glycerol