Published in final edited form as:

Mol Genet Metab. 2011 August ; 103(4): 323–329. doi:10.1016/j.ymgme.2011.04.013.

Ammonia control in children with urea cycle disorders (UCDs); Phase 2 comparison of sodium phenylbutyrate and glycerol phenylbutyrate[☆]

Uta Lichter-Konecki^{a,*}, G.A. Diaz^b, J.L. Merritt II^c, A. Feigenbaum^d, C. Jomphe^e, J.F. Marier^e, M. Beliveau^e, J. Mauney^f, K. Dickinson^g, A. Martinez^g, M. Mokhtarani^g, B. Scharschmidt^g, and W. Rhead^h

^a Children's National Medical Center, 111 Michigan Ave NW, Washington, DC, 20010, USA

^b Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY, USA

^c Seattle Children's Hospital, 4800 Sand Point Way NE, Seattle, WA, USA

^d The Hospital for Sick Children, 555 University Avenue, Toronto Ontario, Canada

^e Pharsight, Montreal, 2000 Peel St., Suite 570, Quebec, Canada

^f Chiltern, 2520 Independence Blvd., Ste. 202, Wilmington NC, USA

^g Hyperion Therapeutics, Inc., 601 Gateway Blvd., Ste. 200, South San Francisco, CA, USA

^h Pediatrics, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI, USA

Abstract

Twenty four hour ammonia profiles and correlates of drug effect were examined in a phase 2 comparison of sodium phenylbutyrate (NaPBA) and glycerol phenylbutyrate (GPB or HPN-100), an investigational drug being developed for urea cycle disorders (UCDs).

Study Design—Protocol HPN-100-005 involved open label fixed-sequence switch-over from the prescribed NaPBA dose to a PBA-equimolar GPB dose with controlled diet. After 7 days on NaPBA or GPB, subjects underwent 24-hour blood sampling for ammonia and drug metabolite levels as well as measurement of 24-hour urinary phenylacetylglutamine (PAGN). Adverse events (AEs), safety labs and triplicate ECGs were monitored.

Results—Eleven subjects (9 OTC, 1 ASS, 1 ASL) enrolled and completed the switch-over from NaPBA (mean dose=12.4 g/d or 322 mg/kg/d; range=198–476 mg/kg/d) to GPB (mean dose=10.8

[☆]ClinicalTrials.gov identifier: NCT00947544.

* Corresponding author at: Department of Pediatrics, George Washington Univ. Medical Center, Division of Genetics and Metabolism, Children's National Medical Center, 111 Michigan Ave NW, Washington, DC 20010, USA. ; Email: ulichter@cnmc.org (U. Lichter-Konecki).

Conflict of interest statement

K. Dickinson, M. Mokhtarani, A. Martinez, and B.F. Scharschmidt are/were employees of Hyperion at the time of the study. C Jomphe, JF Marier and M. Beliveau are employees of Pharsight, which was paid by Hyperion to perform the PK analyses. J. Mauney is an employee of Chiltern, which was paid by Hyperion to perform the biostatistical analyses. None of the other authors have a financial interest in Hyperion, although payments were made by Hyperion to Children's National Medical Center (U. Lichter-Konecki, PI), Mt. Sinai (G. Diaz, PI), Medical College of Wisconsin (W. Rhead, PI), the Univ. of Toronto (A. Feigenbaum, PI) and Seattle Children's Hospital (JL Merritt II, PI) for services provided in the conduct of the study.

mL or 0.284 mL/kg/d or 313 mg/kg/d; range = 192–449 mg/kg/d). Possibly-related AEs were reported in 2 subjects on NaPBA and 4 subjects on GPB. All were mild, except for one moderate AE of vomiting on GPB related to an intercurrent illness. No clinically significant laboratory or ECG changes were observed. Ammonia was lowest after overnight fast, peaked postprandially in the afternoon to early evening and varied widely over 24 h with occasional values >100 $\mu\text{mol/L}$ without symptoms. Ammonia values were ~25% lower on GPB vs. NaPBA ($p = 0.1$ for ITT and $p < 0.05$ for per protocol population). The upper 95% confidence interval for the difference between ammonia on GPB vs. NaPBA in the ITT population (95% CI 0.575, 1.061; $p = 0.102$) was less than the predefined non-inferiority margin of 1.25 and less than 1.0 in the pre-defined per-protocol population (95% CI 0.516, 0.958; $p < 0.05$). No statistically significant differences were observed in plasma phenylacetic acid and PAGN exposure during dosing with GPB vs. NaPBA, and the percentage of orally administered PBA excreted as PAGN (66% for GPB vs. 69% for NaPBA) was very similar. GPB and NaPBA dose correlated best with urinary-PAGN.

Conclusions—These findings suggest that GPB is at least equivalent to NaPBA in terms of ammonia control, has potential utility in pediatric UCD patients and that U-PAGN is a clinically useful biomarker for dose selection and monitoring.

Keywords

Urea cycle disorders; Ammonia scavengers; Hyperammonemia; Phenylacetate; Sodium phenylbutyrate; Glycerol phenylbutyrate

1. Introduction

Urea cycle disorders (UCDs), which comprise 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].

Batshaw, Brusilow and coworkers introduced the concept of alternative pathway pharmacological therapy for UCDs [4,6,7]. These pioneering studies led to the availability of sodium phenylbutyrate (NaPBA), 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 involving deficiencies of carbamylphosphate synthetase (CPS), ornithine transcarbamylase (OTC), or argininosuccinic acid synthetase (AS) and lowers ammonia by enhancing excretion of waste nitrogen in the form of phenylacetylglutamine (PAGN). Despite the fact that alternative pathway therapy with NaPBA has been utilized for over two decades, there are little systematically collected data available pertaining to fasting and postprandial ammonia levels or correlates of drug effect in pediatric patients [8]. GPB is an investigational agent being developed for UCDs. Like NaPBA, it contains phenylbutyric acid (PBA), a pro-drug that is converted via β -oxidation to the active moiety, phenylacetic acid (PAA), which combines with glutamine to form PAGN that is excreted in the urine. However, unlike NaPBA which is a salt, GPB is a pre-pro-drug that contains no sodium and consists of three molecules of PBA joined to glycerol in ester linkage. It is hydrolyzed in the small intestine by pancreatic lipases to release PBA and

glycerol, PBA is absorbed more slowly than when administered as NaPBA, and the glycerol is presumably digested like dietary glycerol consumed in the form of long chain triglycerides [8–10].

2. Materials and methods

2.1. Study design and treatments

This phase 2, open-label, fixed sequence, switch-over study enrolled pediatric patients ages 6 or above being treated with NaPBA for a UCD (confirmed via enzymatic, biochemical or genetic testing). Major exclusion criteria included liver transplant, hypersensitivity to PBA, PAA or PAGN, clinically significant laboratory abnormalities or ECG findings, or conditions or medications that could affect ammonia levels.

Enrolled subjects received NaPBA for at least 7 days, three times daily with meals at the dose level prescribed by the investigator. On the last day of NaPBA treatment they were admitted to a study unit and underwent 24-hour blood sampling for pharmacokinetic (PK) and ammonia measurements. Subjects were then switched to GPB at the PBA molar equivalent of their prescribed NaPBA dose. Initiation of GPB dosing was done under observation in an appropriately monitored setting and subjects were discharged after they were deemed clinically stable. After 7 days of treatment with GPB subjects were re-admitted to the research unit for 24-hour PK and ammonia monitoring, after which they were offered enrollment into a long-term extension study. Only the results of the switch-over part of the protocol are reported here.

Subjects received dietary counseling and remained on their prescribed amount of dietary protein throughout the study. Diet was carefully monitored on study days 7 and 14 during the 24-hour blood sampling as well as for at least 3 days prior to each visit via diaries. Subjects also were queried at the end of the study with respect to their preference for NaPBA or GPB. Compliance with study drug was assessed by daily recording of missed doses by the subject and monitoring drug accountability records and inspection of the returned bottles and vials. Safety was assessed through standard safety laboratory tests, physical exams, serial triplicate ECG, and collection of adverse events. Efficacy was assessed by serial measurement of venous ammonia. An independent Data and Safety Monitoring Board (DSMB) was chartered to oversee the conduct of the study and an interim analysis of safety, ammonia, and PK data was conducted after 6 subjects completed the study.

2.2. Biochemical analyses

NaPBA and GPB metabolites including PBA, PAA, and PAGN were measured by validated liquid chromatography tandem mass spectrometry methods at the bioanalytical laboratory, Quest Pharma Services. Venous ammonia was measured by the accredited hospital laboratory at each site; plasma amino acids were measured by Baylor Medical Genetics Laboratories.

2.3. Pharmacokinetic and ammonia sampling

Blood samples for analysis of venous ammonia, intact GPB, and NaPBA and GPB metabolites were collected on the last day of dosing with either NaPBA or GPB at time zero (pre dose and pre-breakfast) and 4, 8, 12, 16, 20 and 24 h post-first dose. Lunch and dinner typically were eaten after the 4 and 8 h collections, respectively. Plasma amino acids were collected at time 0 (fasting) on days 7 and 14. Urine was collected in aliquots of 0–12 h (beginning with the first dose of the day) and 12–24 h.

2.4. Pharmacokinetic analyses

Pharmacokinetic parameters of PBA, PAA and PAGN in plasma and urine were calculated using a validated version of WinNonlin® Enterprise (Version 5.2). Statistical analyses were performed using WinNonlin v.5.2 (LinMix Module). Plasma PK parameters, including mean and coefficient of variation (standard deviation [SD], expressed as a percentage of the mean), were calculated using actual time-concentration profiles for each subject and included the following: Area under the concentration vs. time curve from time 0 (pre-dose) to 24 h (AUC_{0-24}), calculated using the linear trapezoid rule, maximum plasma concentration at steady state ($C_{max_{ss}}$), minimum plasma concentration at steady state ($C_{min_{ss}}$), time of maximum plasma concentration at steady state ($T_{max_{ss}}$), and apparent clearance at steady state (CL_{ss}/F) (calculated as $Dose/AUC_{0-24}$ over the bioavailable fraction [F]). The amount of PAGN excreted in urine over 24 h was calculated by multiplying urine volume with urinary concentrations. Summary tables and figures were generated using WinNonlin® AutoPilot™ (Version 1.1.1), a configurable software application that works with WinNonlin®, and third-party reporting tools, including SigmaPlot® versions 9.01 and 10.1 and Microsoft® Office Word and Excel 2003 and 2007.

2.5. Ammonia analyses

The primary efficacy endpoint for the switch over phase of the study was 24-hour ammonia AUC ($NH_3_{24-hour AUC}$), calculated based on the sequence of ammonia concentrations outlined above. An imputation algorithm was prespecified in the statistical analysis plan to allow for calculation of $NH_3_{24-hour AUC}$ for subjects with missing ammonia values.

2.6. Efficacy endpoints and statistical analyses

The primary efficacy endpoint was predefined as comparison of $NH_3_{24-hour AUC}$ on the last day of NaPBA treatment with the last day of GPB treatment. Secondary efficacy endpoints included the maximum ammonia concentrations and percentage of abnormal ammonia values on the last day of treatment with NaPBA vs. GPB. All subjects who received any amount of both study medications were included in the intention-to-treat (ITT) population, which was the primary population for analysis of efficacy and pharmacokinetic parameters. A per-protocol population was also prospectively defined as all subjects from the ITT population who (a) exhibited 80% compliance with study medication during inpatient stays (days 7 and 14), (b) did not use sodium benzoate within 48 h prior to days 7 and 14, (c) differed with respect to protein intake on study days 7 and 14 by 50%, and (d) had calculable ammonia AUC (as defined above) for both days 7 and 14.

Non-inferiority analysis of GPB to NaPBA with respect to ammonia control was prospectively defined. An analysis of variance (ANOVA) model for the natural log-transformed $\text{NH}_3_{24\text{-hour}}$ AUC was constructed with factors for treatment as a fixed effect and subject as a random effect. The 90% CI for the difference between GPB and NaPBA means (GPB minus NaPBA) on the natural log scale was constructed using the least square means from the ANOVA model. The difference and lower and upper confidence interval of $\text{NH}_3_{24\text{-hour}}$ AUC values were exponentiated to express the results as geometric means, ratio of geometric means, and corresponding CI on the original scale. Non-inferiority was to be concluded if the upper bound of the 90% CI was less than or equal to 1.25. Correlates of drug dose were determined for both NaPBA and GPB using Spearman rank-order correlations. A superiority analysis was predefined for the per-protocol population.

3. Results

3.1. Patient demographics, disposition and compliance

Eleven subjects (age range 6–17; 1 male and 10 females) enrolled and all 11 subjects completed the protocol defined study procedures. As defined by the DSMB Charter, enrollment was temporarily paused after six subjects completed the switchover portion of the study pending DSMB approval to complete enrollment. Subject demographics are summarized in Table 1. One subject each had argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) deficiency; the remaining 9 subjects had ornithine transcarbamylase (OTC) deficiencies. Six subjects had neonatal or infantile onset and the remainder had later onset UCD. NaPBA had been prescribed for an average (SD) of 74.7 (48.2) months at an average (SD) NaPBA dose of 12.4 (4.4) g/d (equivalent to an average of 322 mg/kg/d or 10.2 g/m²) and received the PBA equivalent dose of GPB (average (SD)=10.8 mL, equivalent to an average dose of 0.284 mL/kg/d or 313 mg/kg/d). Five subjects received NaPBA tablets and 6 received NaPBA powder during the study, and 2 subjects who received NaPBA via an NG tube took GPB orally during the study. Compliance with treatment was excellent; >98% of all scheduled doses of either NaPBA or GPB were apparently taken based on monitoring of vials and bottles.

Dietary protein prescription at baseline among the 11 subjects (mean, SD) was 0.75±0.29 g/kg/d (27.7±9.48 g/day). Compliance on study with diet was less uniform than compliance with respect to study drug. While two subjects showed >50% variance in protein intake on days 7 vs. 14 and were excluded from the per protocol analysis, overall total mean protein intake was very similar on NaPBA and GPB (0.64±0.35 g/kg/d or 24.35 g/d and 0.61±0.16 g/kg/d or 23.98 g/d on days 7 and 14 respectively; Table 4), as was the distribution of dietary protein throughout the day with both GPB and NaPBA at breakfast (4.96±2.497 and 5.46±2.943 g, respectively), lunch (6.41±4.759 and 5.98±5.332 g, respectively), and dinner (6.74±4.653 and 5.71±5.197 g, respectively).

3.2. Safety and tolerability

During the switch over period of the study all reported AEs were categorized as mild except one episode of vomiting graded as moderate on GPB, which resolved and was attributed to intercurrent illness and deemed unrelated to study drug (Table 2). There were no episodes of

hyperammonemic crisis, predefined as blood ammonia exceeding 100 $\mu\text{mol/mL}$ plus signs or symptoms of hyperammonemia on either NaPBA or GPB. Each patient was asked by the investigator or representative to evaluate his or her drug preference on day 14 of the study; all 11 subjects stated a preference for GPB.

3.3. Pharmacokinetic analyses

All 11 patients were considered evaluable for the PK analyses, as were all measurable concentration values. Values below the lower limit of quantification were treated as zero. Individual plasma metabolite values varied widely for both NaPBA and GPB whether drawn at peak (12 h), trough (24 h; selected over time zero because of monitored dosing) or throughout the course of the day (0–24 h) (Table 3). Intact GPB was not detectable in plasma.

Plasma PK parameters of PBA, PAA and PAGN and urinary PK parameters of PAGN are summarized in Table 4 and the 24-hour concentration profiles are depicted in Fig. 1. Mean systemic PBA exposure (AUC_{0-24}) following GPB administration was ~ 2.7 -fold higher ($p < 0.01$) than that observed with NaPBA. However, when compared with NaPBA administration, there was less variability in plasma PBA levels during dosing with GPB, as reflected by the coefficient of variation for AUC, C_{max} and C_{min} (Table 4). Mean (AUC_{0-24}) systemic PAA and PAGN exposure during dosing with GPB as compared with NaPBA did not differ significantly, albeit also with directionally greater values on GPB as compared with NaPBA. Minimum (C_{min}) values of PAA were significantly greater during GPB as compared with NaPBA dosing.

Similar to plasma, all urines were judged analyzable. One subject had no urine sample for the first interval and another did not void during the 12–24 h post-dose collecting interval, therefore their total PAGN amount excreted over 24 h ($\text{U-PAGN}_{24\text{-hour Excr}}$) was adjusted as prospectively detailed in pharmacokinetic analysis plan. 24-hour PAGN excretion following GPB treatment was very similar to that observed for NaPBA (66% vs. 69% recovery of PBA as urinary PAGN), although peak urinary PAGN occurred later in the day during GPB treatment as compared with NaPBA treatment (percentage of total output from 0 to 12 and 12 to 24 h approximately 45% and 55% for GPB vs. 57% and 43% for NaPBA).

3.4. Plasma amino acids

Three patients had glutamine values above the normal range (266–746 $\mu\text{mol/L}$) on GPB as compared with 6 patients on NaPBA. Mean [SD] plasma glutamine levels were non-statistically significantly lower on GPB (650.3 [187.3] $\mu\text{mol/L}$) vs. NaPBA (725.1 [204.2] $\mu\text{mol/L}$). Mean [SD] plasma branched chain amino acid levels were similar with NaPBA vs. GPB (isoleucine=34.1 [20.2] vs. 38.6 [9.2], leucine=64.0 [38.3] vs. 68.0 [16.9], and valine=134.5 [65.36] vs. 112.0 [36.36] on NaPBA and GPB, respectively).

3.5. Blood ammonia

Ammonia levels varied widely, increased several fold during the day, peaking at around 8–12 h, and in four samples (3 during NaPBA and 1 during GPB treatment, respectively) exceeded 100 $\mu\text{mol/L}$ in the absence of clinical symptoms. Only 2 of 154 blood ammonia

values were missing; all NH_3 _{24-hour} AUC values were calculable and no imputation was required. Average ammonia values tended to be lower on GPB than on NaPBA assessed as NH_3 _{24-hour} AUC, average blood ammonia, average $\text{C}_{\text{max,ss}}$ or the percentage of values above the upper limit of normal (as per normal values at the respective study site; range 29–54 $\mu\text{mol/L}$) (Table 4, Figs. 2 and 3), although these differences did not achieve statistical significance. GPB was determined to be non-inferior to NaPBA with respect to ammonia control based on the pre-specified analysis, as the upper boundaries of both the 90% (0.609, 1.002) and 95% (0.575, 1.061) confidence intervals fell below 1.25. Analysis of ammonia control in the pre-specified per-protocol population demonstrated significantly lower ammonia (623.1 vs. 897.2), assessed as NH_3 _{24-hour} AUC on GPB as vs. NaPBA ($p < 0.05$). This per-protocol analysis on 9 of 11 subjects excluded the two with a variance of over 50% in dietary protein intake on days 7 and 14.

3.6. Effect of age on PK and ammonia during GPB dosing

PK parameters during steady state dosing with GPB analyzed separately for subjects ages 6–11 ($n=7$) vs. 12–17 ($n=4$) were similar, as were mean (SD) ammonia values assessed as NH_3 _{24-hour} AUC (Table 5).

3.7. Correlates of drug dose and ammonia

Drug dose correlated directly and most consistently and strongly with urinary PAGN for both NaPBA and GPB treatments ($r=0.866$; $p < 0.0001$ for both NaPBA and GPB combined) (Table 6). Drug dose did not correlate with plasma PBA levels assessed as AUC_{0-24} . Drug dose did correlate, albeit less strongly and consistently, with plasma PAGN and PAA, assessed as AUC_{0-24} . Ammonia assessed as NH_3 _{24-hour} AUC correlated neither with blood levels of PBA, PAA or PAGN nor with urinary PAGN output. Blood ammonia C_{max} correlated positively with glutamine, although the correlation was modest ($r=0.25$) and did not achieve significance in this small sample size.

4. Discussion

No clinically important safety issues were identified during GPB dosing and tolerability was satisfactory. No hyperammonemic crises occurred during either NaPBA or GPB treatment. Plasma PBA exposure was ~2.7 times greater ($p < 0.01$) during dosing with GPB as compared with NaPBA, whereas plasma PAA and PAGN exposure differed by ~25% and ~35%, respectively. Although these differences in PAA and PAGN on GPB and NaPBA were not statistically significant ($p > 0.1$), the sample size is small.

Blood ammonia levels peaked postprandially in the evening and varied widely over 24 h on both NaPBA and GPB, with about one-third of ammonia values during NaPBA dosing exceeding the normal range including occasional values exceeding 100 $\mu\text{mol/L}$. The results suggest that ammonia values are of greatest utility if drawn at a constant time in relation to meals.

Ammonia values tended to be lower on GPB as compared with NaPBA, whether assessed as NH_3 _{24-hour} AUC, mean values, maximal values, or the percentage of abnormal values. Analysis of the per-protocol population as pre-defined, including pre-defined compliance

with diet, demonstrated significantly lower ammonia values on GPB. A pre-defined non-inferiority ‘intention-to-treat’ analysis of GPB as compared with NaPBA in all 11 patients indicated that non-inferiority of GPB was achieved.

The finding of directionally lower ammonia values on GPB as compared with NaPBA is similar to that observed in a phase 2 study of adult UCD patients [8]. This finding is not explained by the percentage of the administered PBA which participates in waste nitrogen scavenging as measured by urinary output of PAGN, since the percentage conversion was similar for NaPBA and GPB (69% vs. 66%) in the present study, as it was previously in adults [8]. It is also not explained by differences in total protein intake, which was nearly identical on GPB (Day 14; 23.98 ± 9.891 g/d or 0.61 ± 0.16 g/kg/d) and NaPBA (Day 7; 24.35 ± 12.445 g/d or 0.64 ± 0.35 g/kg/d).

One possible explanation for the directionally lower ammonia values is the generally higher plasma metabolite levels observed during GPB dosing. As summarized in Table 4, GPB treatment resulted in higher plasma PBA levels overall, including higher peak and trough levels, and similar differences in favor of higher plasma levels for PAA and PAGN. Plasma PAA levels were better sustained and directionally higher over night, as manifested by higher trough levels and this could be causally related to the directionally lower ammonia levels during GPB dosing.

The PAA levels are of interest in that neurological toxicity has been reported in cancer subjects administered high doses of PAA intravenously; this toxicity was associated with PAA blood levels ranging from ~499 to 1285 $\mu\text{g/mL}$ [11,12]. However, PAA blood levels observed during steady state dosing with both NaPBA and GPB were well below the levels reported by Thibault to be associated with neurological symptoms (Table 4).

Another possible explanation for and contributing factor to the ammonia findings may be slower gastrointestinal absorption of PBA when delivered as GPB. This is supported by prior PK/PD modeling (10) and, in the present study, by the proportion of PAGN excreted during the 0–12 and 12–24 h time periods (45% and 55% for GPB vs. 57% and 43% for NaPBA). A slower gastrointestinal absorption of PBA is also indicated by lesser variation in PBA concentrations during GPB dosing in this study (Table 4).

While the number of subjects is small, ammonia and PK findings appeared to be generally similar during steady state GPB among subjects ages 6–11 and 12–17, suggesting that GPB metabolism and effect is not dependent on age, per se, within this range (Table 5).

The present study underscores the difficulty clinicians face in making decisions regarding drug dosing based on blood ammonia. Even under the controlled conditions of the present trial, including excellent compliance with diet and study drug in patients whose ammonia values were viewed as well controlled by their physicians prior to enrollment, ammonia values on average varied more than 10-fold over the course of the day. This observation suggests that random values for blood ammonia are of limited utility and that ammonia levels should be drawn at a constant time in relation to meals and medication for monitoring of treatment. Blood levels of metabolites are also problematic with respect to therapeutic monitoring. As summarized in Tables 3 and 4 and depicted in Fig. 1, blood levels of PBA,

PAA and PAGN all varied widely over the course of the day. Levels at individual time points and even peak or trough levels varied considerably among these patients (Table 3), suggesting that therapeutic metabolite blood levels when collected randomly or at peak or trough time points are difficult if not impossible to define. Although PAA and/or PAGN exposure assessed as 24 h area under the curve and measured with 8 samples over 24 h did correlate with dose, the correlation was weak and inconsistent and 24-hour sampling is impractical in routine practice.

Urinary output of PAGN, in contrast to blood levels of drug metabolites, shows promise as a biomarker of dose selection and compliance monitoring in pediatric UCD patients and is practical to perform in routine clinical practice. This makes theoretical sense, since PAGN output is stoichiometrically related to waste nitrogen scavenging, and PAGN output, measured over 24 h, correlated strongly and positively with drug dose ($R=0.909$; $p<0.001$ for GBP and $R=0.753$; $p=0.007$ for NaPBA).

Many metabolic specialists use glutamine levels to monitor treatment and adjust drug doses. Among the subjects in this study, who were viewed as well-controlled under the supervision of a metabolic specialist at entry, glutamine levels decreased, although not statistically significantly, after switching from NaPBA to GBP. Glutamine levels also correlated modestly and positively with ammonia. However, the correlation did not achieve statistical significance and needs evaluation in a larger study population.

Finally, the satisfactory safety and short-term ammonia control observed during GBP dosing as compared with NaPBA are collectively encouraging with respect to the potential utility of GBP as a therapeutic option for UCD patients and indicate that further studies, including longer-term studies of safety and ammonia control, are warranted.

Acknowledgments

This study was sponsored by Hyperion Therapeutics. The authors acknowledge the clinical research staffs at Children's National Medical Center (Kara Simpson), the Medical College of Wisconsin (Patricia Chico, MA), the Mt Sinai School of Medicine (Javier Delgado, Christina Guzman), the Hospital for Sick Children (Mohammad Hussain) and the Seattle Children's Hospital (Linnea Brody). The work was supported in part by NIH CTSA Grant UL1RR029887 to Mount Sinai School of Medicine (Dr. Diaz) and NIH CTSA Grant UL1 RR025014-XX to the University of Washington, Institute of Translational Health Science (Dr. Merritt), MO1-RR-020359-04 to Children's National Medical Center, and the Urea Cycle Disorders Consortium, a Rare Disease Clinical Research Center (9U54HD061221 NIH, NICHD) and the O'Malley Foundation.

Abbreviations

ASL	argininosuccinate lyase deficiency
ASS	argininosuccinate synthetase deficiency
AUC₀₋₂₄	24 h 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
NH₃24-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-PAGN_{24-hour} Excr	PAGN excreted in urine over 24 h

References

1. Brusilow SW, Maestri NE. Urea cycle disorders: diagnosis, pathophysiology, and therapy. *Adv. Pediatr.* 1996; 43:127–170. [PubMed: 8794176]
2. Summar ML, Dobbelaere D, Brusilow S, Lee B. Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. *Acta Paediatr.* 2008; 97:1420–1425. [PubMed: 18647279]
3. Tuchman M, Lee B, Lichter-Konecki U, Summar ML, Yudkoff M, Cederbaum SD, Kerr D, Diaz G, Seashore M, Lee H, McCarter R, Krischer J, Batshwa M. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. *Mol. Genet. Metab.* 2008; 94:397–402. [PubMed: 18562231]
4. Batshaw ML, Brusilow S, Waber L, Blom W, Brubakk AM, Burton BK, Cann HM, Kerr D, Mamunes P, Matalon R, Myerberg D, Schafer IA. Treatment of inborn errors of urea synthesis: activation of alternative pathways of waste nitrogen synthesis and excretion. *N. Engl. J. Med.* 1982; 306:1387–1392. [PubMed: 7078580]
5. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *N. Engl. J. Med.* 2007; 356:2282–2292. [PubMed: 17538087]
6. Brusilow SW. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. *Pediatr. Res.* 1991; 29:147–150. [PubMed: 2014149]
7. Brusilow SW, Jindalstein J. Restoration of nitrogen homeostasis in a man with ornithine transcarbamylase deficiency. *Metabolism.* 1993; 42:1336–1339. [PubMed: 8412748]
8. Lee B, Rhead W, Diaz Test GA. Phase 2 comparison of a novel ammonia scavenging agent with sodium phenylbutyrate in patients with urea cycle disorders: safety, pharmacokinetics and ammonia control. *Mol. Genet. Metab.* 2010; 100:221–228. [PubMed: 20382058]
9. McGuire BM, Zupanets I, Lowe ME, Sypliyviy V, Monteleone J, Gargosky S, Nash C, Dickenson K, Martinez A, Mokhtarani M, Scharschmidt BF. Pharmacology and safety of glycerol phenylbutyrate in healthy adults and adults with cirrhosis. *Hepatology.* 2010; 51:2077–2085. [PubMed: 20512995]
10. Monteleone JPR, Dykstra KH, Lee B, McGuire BM, Berry S, Diaz G, Rhead W, Sypliyviy V, Zupanets I, Gargosky S, Martinez A, Mokhtarani M, Scharschmidt BF. HPN-100, a novel

investigational ammonia scavenging agent, demonstrated unique behavior based on pharmacokinetic/pharmacodynamic (PK/PD) modeling. *Mol. Genet. Metab.* 2010; 99:232.

11. Thibault A, Cooper M, Figg W, Venzon D, Sartor A, Tompkins A, Weinberger M, Headlee D, McCall N, Samid D, Myers C. A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. *Cancer Res.* 1994; 54:1690–1694. [PubMed: 8137283]
12. Thibault A, Samid D, Cooper MR, Figg WD, Tompkins AC, Patronas N, Headlee DJ, Kohler DR, Venzon DJ, Myers CE. Phase I study of phenylacetate administered twice daily to patients with cancer. *Cancer.* 1995; 75:2932–2938. [PubMed: 7773944]

Fig. 1.

Plasma phenylbutyric acid (PBA; top panel), phenylacetic acid (PAA; middle panel) and phenylacetylglutamine (PAGN; bottom panel) were measured for 24 h following one week of dosing with either sodium phenylbutyrate (NaPBA) or glycerol phenylbutyrate (GPB) and are displayed as median $\pm$ SD. Times 0 and 24 h correspond to just prior to dosing and breakfast.

Fig. 2.

Twenty Four Hour Ammonia Values on Sodium Phenylbutyrate and Glycerol Phenylbutyrate. Venous ammonia was measured for 24 h following one week of dosing with either sodium phenylbutyrate (NaPBA; continuous line) or glycerol phenylbutyrate (GPB; dotted line) in 11 pediatric subjects. The top panel depicts mean (SE) ammonia concentrations over 24 h. The bottom panel depicts overall ammonia values, where the bottom and top of the 'box' represent the 25th and 75th percentile of all values, the horizontal line within the box represents the mean, the open diamond within the box represents the median, and the top and bottom of the lines correspond to the maximum and minimum observed values, respectively. The open circles above the box for ammonia (24-hours AUC) on GPB represent outliers above the 75th percentile.

Fig. 3.

Venous ammonia in individual subjects following one week of dosing with either sodium phenylbutyrate (NaPBA; left) or glycerol phenylbutyrate (GPB; right). The values shown represent time-normalized area under the curve and are displayed as mean $\pm$ SD. Times 0 and 24 h correspond to just prior to dosing and breakfast.

Table 1

Patient demographics.

Patients completing the study (N = 11)	
Gender [n (%)]	
Male	1 (9.1%)
Female	10 (90.9%)
Age (years) at Screening	
Mean (SD)	10.2 (3.95)
Height (cm)	
Mean (SD)	133.66 (16.900)
Weight (kg)	
Mean (SD)	41.79 (20.135)
UCD Diagnosis [n (%)]	
OTC deficiency ^a	9 (81.8%)
ASS deficiency ^b	1 (9.1%)
ASL deficiency ^c	1 (9.1%)
UCD onset [n (%)]	
Neonatal (0 – <=30 days)	3 (27.3%)
Infantile (>30 days – <=2 years)	3 (27.3%)
Childhood or adult onset (>2 years)	5 (45.5%)
Duration of NaPBA treatment (months)	
Mean (SD)	74.68 (48.220)
Median	76.00
Min, Max	0.5 - 162.0
Type of NaPBA [n (%)]	
Powder ^d	7 (63.6%)
Tablets	4 (36.4%)
NaPBA daily dose (mg/kg/day)	
Mean (SD)	12.41 (4.392)
Median	10.50
Min, Max	8.0–20.0
Subjects with a G-tube	2 (18.2%)
Average prescribed protein intake during study (g/kg/day)	
Mean (SD)	0.75 ± 0.29
Patients treated with L-Citrulline (%)	9 (81.8%)

^a Ornithine transcarbamylase deficiency.^b Argininosuccinate synthetase deficiency.^c Argininosuccinate lyase deficiency.^d One patient was switched from powder to tablets for the study.

Table 2Adverse events ¹.

Preferred term	NaPBA (N = 11)	GPB (N = 11)
Number of subjects with at least one AE	2 (18.2%)	4 (36.4%)
Grade 1	2 (18.2%)	3 (27.3%)
Grade 2	0 (0.0%)	1 (9.1%)
Lymphadenopathy (Grade 1)	1 (9.1%)	0 (0.0%)
Abdominal pain upper (Grade 1)	0 (0.0%)	2 (18.2%)
Vomiting (Grade 2)	0 (0.0%)	1 (9.1%)
Decreased appetite (Grade 1)	1 (9.1%)	0 (0.0%)
Ear infection (Grade 1)	0 (0.0%)	1 (9.1%)
Upper respiratory tract infection (Grade 1)	0 (0.0%)	1 (9.1%)
Cardiac murmur (Grade 1)	1 (9.1%)	0 (0.0%)
Dermatitis contact (Grade 1)	0 (0.0%)	1 (9.1%)

¹ Table reflects number of adverse events reported during 7 days of dosing with sodium phenylbutyrate (NaPBA) and 7 days of dosing with glycerol phenylbutyrate (GPB).

Table 3

Sodium phenylbutyrate (NaPBA) and glycerol phenylbutyrate (GPB) plasma metabolite concentrations (ug/mL) ^{*}.

Time	PBA		PAA		PAGN	
	NaPBA	GPB	NaPBA	GPB	NaPBA	GPB
Trough (24 h)						
Mean ± SD	0.843 ± 1.28	6.43 ± 16.0	0.817 ± 1.03	9.13 ± 18.6	4.71 ± 3.08	27.7 ± 39.2
Range	<1–3.48	<1–54.5	<1–2.84	<1–62.9	<1–7.94	2.63–137
Peak (12 h)						
Mean ± SD	36.8 ± 38.2	74.8 ± 52.6	68.8 ± 47.4	78.6 ± 65.2	72.2 ± 28.5	88.8 ± 33.8
Range	2.28–109	4.14–161	3.43–148	12.1–244	30.8–113	32.0–136
Range of single measurements (0–24 h)						
Range	<1–109	<1–161	<1–148	<1–244	<1–116	2.63–153

^{*} PBA = phenylbutyric acid; PAA = phenylacetic acid; and PAGN = phenylacetylglutamine.

Table 4

PK parameters and ammonia at steady state dosing with sodium phenylbutyrate and glycerol phenylbutyrate (Study days 7 and 14).

PK/PD parameters	Glycerol phenylbutyrate (n = 11)	NaPBA (n = 11)
PBA in plasma mean (CV%)		
AUC ₀₋₂₄ (µg·h/mL)	631 (44.9)	236 (105.2)
Cmax _{ss} (µg/mL)	95.6 (42.0)	37.4 (101.6)
Cmin _{ss} (µg/mL)	1.50 (99.8)	0.366 (171.3)
PAA in plasma mean (CV%)		
AUC ₀₋₂₄ (µg·h/mL)	964 (63.6)	773 (73.3)
Cmax _{ss} (µg/mL)	90.5 (69.1)	75.1 (64.4)
Cmin _{ss} (µg/mL)	2.99 (122.1)	0.674 (130.5)
PAGN in plasma mean (CV%)		
AUC ₀₋₂₄ (µg·h/mL)	1378 (40.2)	1015 (44.7)
Cmax _{ss} (µg/mL)	105 (33.5)	74.8 (37.3)
Cmin _{ss} (µg/mL)	13.1 (64.9)	4.63 (66.4)
PAGN in urine mean (CV%)		
Total excreted 0–24 h (g)	12.5 (56.9)	12.5 (51.3)
Recovery of PBA as PAGN (%) Fe ₀₋₂₄ (%)	66.4 (23.9)	69.0 (23.9)
Ammonia mean (SD)		
AUC (µmol/L)	603.8 (187.92)	814.6 (322.36)
Cmax _{ss} (µmol/L)	47.77 (12.800)	55.66 (21.607)
Total number (%) of ammonia values above ULN *	24 (31.6)	14 (18.4)
Diet		
Actual protein intake (g/d) Mean (SD)	23.98 (9.891)	24.35 (12.445)
Actual protein intake (g/kg/d) Mean (SD)	0.61 (0.16)	0.64 (0.35)
Judged compliant with diet (days 7, 14)	8/11 (72.7%)	9/11 (81.8%)
Difference in ammonia between GPB and NaPBA		
Mean difference of AUC (µmol/L) (SD)	–210.8 (310.89)	
Ratio of Geometric means	0.781	
90% Confidence interval	(0.609, 1.002)	
95% Confidence interval	(0.575, 1.061)	

AUC₀₋₂₄: Area under the concentration from time 0 (pre-dose) to 24 h, Cmax_{ss}:Maximum plasma concentration at steady state, Cmin_{ss}: Minimum plasma concentration at steady state, CV% – coefficient of variation.

* % abnormal ammonia values presented as mean (SD); the denominator is the total number of ammonia values at all time points (76).

Table 5

PK parameters and ammonia at steady state dosing with glycerol phenylbutyrate (Study day 14) by age range.

PK/PD parameters	All subjects (n = 11)	Ages 6–11 (n = 7)	Ages 12–17 (n = 4)
PBA in plasma Mean (CV%)			
AUC _{0–24} (µg·h/mL)	631 (44.9)	678 (46.9)	548 (41.0)
Cmax _{ss} (µg/mL)	95.6 (42.0)	103 (37.6)	83.3 (54.6)
Cmin _{ss} (µg/mL)	1.50 (99.8)	1.37 (106.5)	1.71 (102.3)
PAA in plasma Mean (CV%)			
AUC _{0–24} (µg·h/mL)	964 (63.6)	894 (49.6)	1086 (83.8)
Cmax _{ss} (µg/mL)	90.5 (69.1)	85.9 (44.5)	98.6 (101.3)
Cmin _{ss} (µg/mL)	2.99 (122.1)	1.70 (76.8)	5.26 (104.8)
PAGN in plasma Mean (CV%)			
AUC _{0–24} (µg·h/mL)	1378 (40.2)	1364 (42.4)	1402 (42.2)
Cmax _{ss} (µg/mL)	105 (33.5)	104 (32.0)	106 (41.0)
Cmin _{ss} (µg/mL)	13.1 (64.9)	10.1 (61.7)	18.4 (56.0)
PAGN in urine Mean (CV%)			
Total excreted	12501037 (56.9)	8382508 (36.6)	19708462 (32.6)
0–24 h (µg)			
Ammonia mean (SD)			
AUC (µmol/L)	603.8 (187.92)	639.36 (226.928)	541.64 (81.365)
Cmax _{ss} (µmol/L)	47.77 (12.800)	50.16 (15.035)	43.58 (7.563)

NA: not available.

Table 6

Correlations between dose and plasma PAA, PBA and PAGN, and 24-hour urinary PAGN excretion (U-PAGN) ¹.

		GPB N = 11	NaPBA N = 11	All N = 22
U-PAGN _{24-hour} Excretion (μg)	R	0.909	0.753	0.866
	p	0.0001	0.0074	<.0001
Plasma PAA (μg·h/mL)	R	0.582	0.484	0.498
	p	0.0604	0.1314	0.0184
Plasma PAGN (μg·h/mL)	R	0.627	0.575	0.514
	p	0.0388	0.0640	0.0144
Plasma PBA (μg·h/mL)	R	0.309	0.461	0.211
	p	0.3550	0.1534	0.3465
NH ₃ _{24-hour} AUC (μmol·h/L)	R	-0.409	-0.174	-0.246
	p	0.2115	0.6099	0.2691

¹ Spearman rank-order correlation (R); p value (p).