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## 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 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 μmol/L without symptoms. Ammonia values were ~25% lower on GPB vs. NaPBA ( $p \geq 0.1$  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.

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**Abbreviations:** ASL, argininosuccinate lyase deficiency; ASS, argininosuccinate synthetase deficiency; AUC<sub>0–24</sub>, 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; NH<sub>3</sub><sub>24-hour AUC</sub>, 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<sub>24-hour Excr</sub>, PAGN excreted in urine over 24 h.

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## 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  $\beta$ -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

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

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  $\geq 80\%$  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  $\leq 50\%$ , 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/m<sup>2</sup>) 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  $0.75 \pm 0.29$  g/kg/d ( $27.7 \pm 9.48$  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 ( $0.64 \pm 0.35$  g/kg/d or 24.35 g/d and  $0.61 \pm 0.16$  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 ( $4.96 \pm 2.497$  and  $5.46 \pm 2.943$  g, respectively), lunch ( $6.41 \pm 4.759$  and  $5.98 \pm 5.332$  g, respectively), and dinner ( $6.74 \pm 4.653$  and  $5.71 \pm 5.197$  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

**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 <sup>a</sup>	9 (81.8%)
ASS deficiency <sup>b</sup>	1 (9.1%)
ASL deficiency <sup>c</sup>	1 (9.1%)
UCD onset [n (%)]	
Neonatal (0 - $\leq 30$ days)	3 (27.3%)
Infantile ( $>30$ days - $\leq 2$ 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 <sup>d</sup>	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)	$0.75 \pm 0.29$
Patients treated with L-Citrulline (%)	9 (81.8%)

<sup>a</sup> Ornithine transcarbamylase deficiency.

<sup>b</sup> Argininosuccinate synthetase deficiency.

<sup>c</sup> Argininosuccinate lyase deficiency.

<sup>d</sup> One patient was switched from powder to tablets for the study.

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

**Table 2**  
Adverse events<sup>1</sup>.

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%)

<sup>1</sup> Table reflects number of adverse events reported during 7 days of dosing with sodium

**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.

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.

**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%)		
$AUC_{0-24}$ ( $\mu\text{g} \cdot \text{h/mL}$ )	631 (44.9)	236 (105.2)
$C_{max_{ss}}$ ( $\mu\text{g/mL}$ )	95.6 (42.0)	37.4 (101.6)
$C_{min_{ss}}$ ( $\mu\text{g/mL}$ )	1.50 (99.8)	0.366 (171.3)
PAA in plasma mean (CV%)		
$AUC_{0-24}$ ( $\mu\text{g} \cdot \text{h/mL}$ )	964 (63.6)	773 (73.3)
$C_{max_{ss}}$ ( $\mu\text{g/mL}$ )	90.5 (69.1)	75.1 (64.4)
$C_{min_{ss}}$ ( $\mu\text{g/mL}$ )	2.99 (122.1)	0.674 (130.5)
PAGN in plasma mean (CV%)		
$AUC_{0-24}$ ( $\mu\text{g} \cdot \text{h/mL}$ )	1378 (40.2)	1015 (44.7)
$C_{max_{ss}}$ ( $\mu\text{g/mL}$ )	105 (33.5)	74.8 (37.3)
$C_{min_{ss}}$ ( $\mu\text{g/mL}$ )	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 ( $\mu\text{mol/L}$ )	603.8 (187.92)	814.6 (322.36)
$C_{max_{ss}}$ ( $\mu\text{mol/L}$ )	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 ( $\mu\text{mol/L}$ ) (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)

$AUC_{0-24}$ : Area under the concentration from time 0 (pre-dose) to 24 h,  $C_{max_{ss}}$ : Maximum plasma concentration at steady state,  $C_{min_{ss}}$ : Minimum plasma concentration at steady state, CV% – coefficient of variation.

\* % abnormal ammonia values presented as mean (SD); the denominator is the total

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 ( $U\text{-PAGN}_{24\text{-hour Excr}}$ ) 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

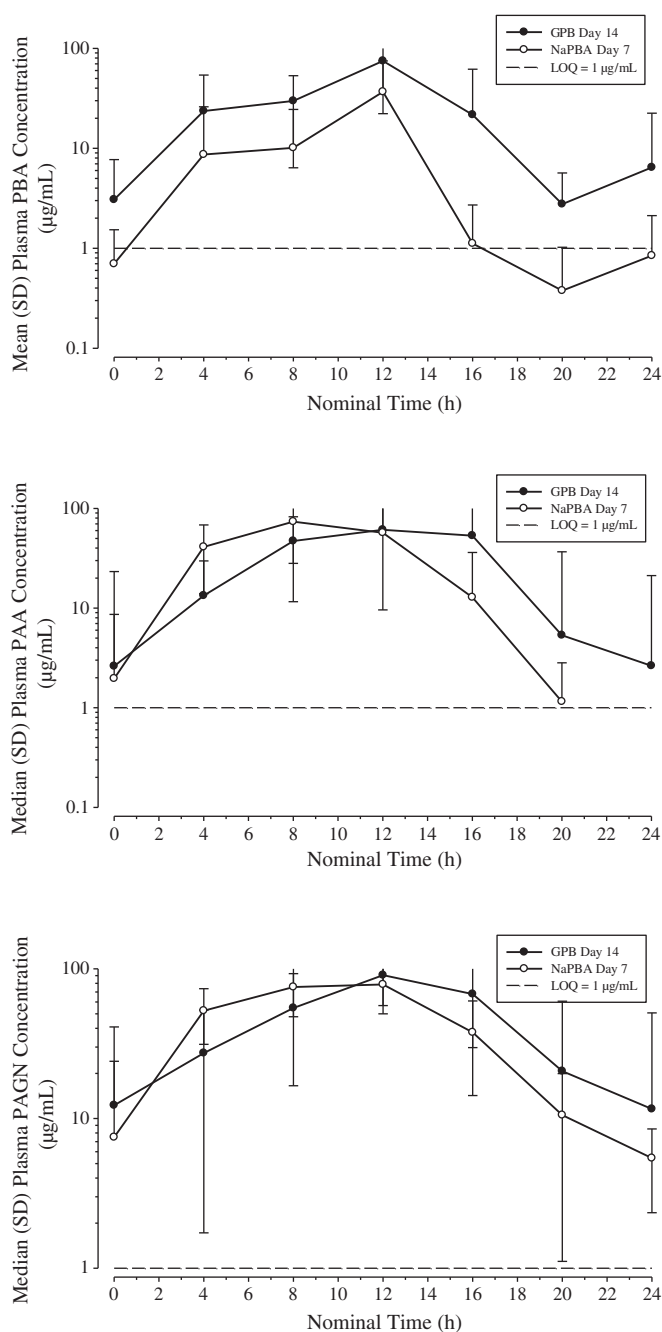
Three patients had glutamine values above the normal range (266–746  $\mu\text{mol/L}$ ) on GPB as compared with 6 patients on NaPBA. Mean [SD] plasma glutamine levels were non-statistically significantly lower on GPB (650.3 [187.3]  $\mu\text{mol/L}$ ) vs. NaPBA (725.1 [204.2]  $\mu\text{mol/L}$ ). 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  $\text{NH}_3_{24\text{-hour AUC}}$  values were calculable and no imputation was required. Average ammonia values tended to be lower on GPB than on NaPBA assessed as  $\text{NH}_3_{24\text{-hour AUC}}$ , average blood ammonia, average  $C_{max_{ss}}$  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  $\text{NH}_3_{24\text{-hour AUC}}$  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

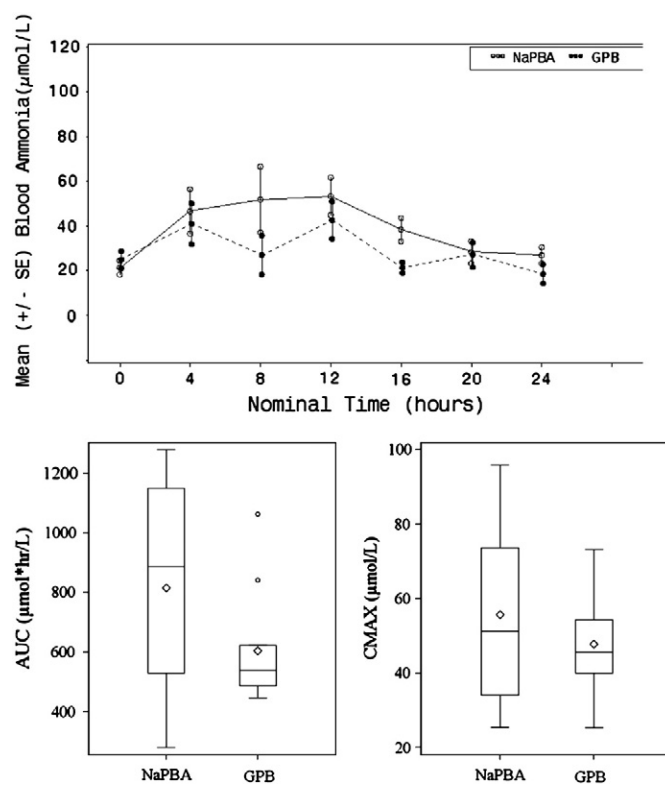
PK parameters during steady state dosing with GPB analyzed separately for subjects ages 6–11 ( $n = 7$ ) vs. 12–17 ( $n = 4$ ) were similar,



**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.

### 3.7. Correlates of drug dose and ammonia

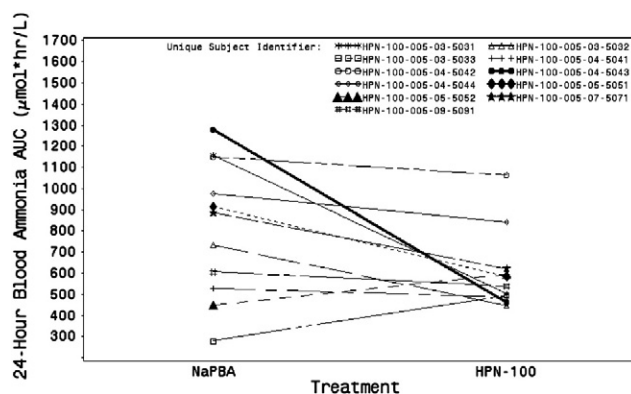
Drug dose correlated directly and most consistently and strongly with urinary PAGN for both NaPBA and GPB treatments ( $r = 0.866$ ;  $p < 0.0001$  for both NaPBA and GPB combined) (Table 6). Drug dose did not correlate with plasma PBA levels assessed as  $AUC_{0-24}$ . Drug dose did correlate, albeit less strongly and consistently, with plasma PAGN and PAA, assessed as  $AUC_{0-24}$ . Ammonia assessed as  $NH_3_{24\text{-hour}}$  AUC correlated neither with blood levels of PBA, PAA or PAGN nor with urinary PAGN output. Blood ammonia Cmax correlated positively with glutamine, although the correlation was modest



**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.

### 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  $\sim 2.7$  times greater ( $p < 0.01$ ) during dosing with GPB as compared with NaPBA, whereas plasma PAA and PAGN exposure differed by



**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

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