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Glycerol Phenylbutyrate in Patients With Cirrhosis and Episodic Hepatic Encephalopathy: A Pilot Study of Safety and Effect on Venous Ammonia Concentration

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Abstract

Glycerol tri-(4-phenylbutyrate) (glycerol phenylbutyrate, GPB, HPN-100) mediates waste nitrogen excretion through conjugation with glutamine to form phenylacetylglutamine which is excreted in urine. This pilot study was performed to assess tolerability and effect on venous ammonia concentration in patients with cirrhosis and hepatic encephalopathy (HE). Patients underwent one week of 6 mL (6.6 g) twice daily (BID). GPB dosing followed by 3 weeks of 9 mL (9.9 g) BID dosing and underwent repeated blood sampling for ammonia concentration and pharmacokinetics. Fifteen patients were enrolled. Ammonia concentrations were lowest after overnight fast and increased post-prandially. Fasting ammonia concentrations were lower on GPB compared to baseline, with a decrease on the eighth day of 6 mL BID dosing to 45.4 (27.9) μmol/L (ULN ~48 μmol/L) ($P < .05$). Nine milliliters BID yielded similar lowering but was associated with more adverse events and higher phenylacetate (PAA) plasma concentrations (PAA C_{max} of 144 [125] vs. 292 [224] μg/mL on 6 and 9 mL, respectively). GPB dosed at 6 mL BID lowered fasting ammonia levels in cirrhotic patients with HE as compared with baseline, was better tolerated than 9 mL BID, and is appropriate for further evaluation in patients with cirrhosis and episodic HE.

Keywords

ammonia, cirrhosis, hepatic encephalopathy, phenylacetylglutamine, phenylbutyrate

The pathogenesis of hepatic encephalopathy (HE) is widely assumed to involve the systemic accumulation of ammonia resulting from portal-systemic shunting and intrinsically impaired hepatic conversion of ammonia to urea, and current treatment with poorly absorbable disaccharides and antibiotics is based on this premise.[1–4] However, the implication of ammonia in the pathogenesis of HE is based largely on correlative studies.[5] Moreover, current treatment is not ammonia selective, and rifaximin, which was recently approved for the treatment of episodic HE, was reported to have only a modest effect on ammonia.[6,7] Glycerol phenylbutyrate (GPB; glyceryl tri-(4-phenylbutyrate); also referred to as HPN-100) is an oral agent approved for urea cycle disorders (UCD) and an investigational agent under development for HE.[8–10] It is a pro-drug of phenylbutyric acid (PBA), currently marketed as sodium phenylbutyrate (BUPHENYL), for the treatment of UCDs (Figure 1) . GPB, which consists of 3 molecules of PBA joined to glycerol via ester linkage, is a pale-yellow, odorless and nearly tasteless sodium-free liquid.

As a prelude to a randomized, blinded, placebo controlled trial in patients with episodic HE, the present open label study was performed. The major objective of the study was to determine whether GPB doses of

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6 or 9 mL BID, which correspond to the mid and upper end of the range commonly used in adult UCD patients, exhibit satisfactory safety and sufficient evidence of activity, assessed as ammonia lowering, to warrant further study.

Materials and Methods

Study Design and Treatments

This primary objectives of this open label, phase 2a study (Protocol HPN-100-008, Part A) were to evaluate the safety, tolerability and activity, assessed as ammonia concentration lowering, of 6 and 9 mL BID GPB doses in patients with cirrhosis and episodic HE and to determine whether either dose was suitable for further study. Patients received 6 mL of GPB (equivalent to 6.6 g of GPB) BID for 7 days followed by 9 mL (equivalent to 9.9 g of GPB) BID for 21 days. GPB was administered orally with morning and evening meals. Patients continued to receive their standard of care treatment for HE.

Patient Population

Adult patients with Child-Pugh B or C cirrhosis who experienced 2 or more documented episodes of West Haven Grade ≥ 2 HE in the prior 6 months, at least 1 of which occurred within 3 months of randomization, were eligible. Patients were stable and judged by the investigator to be in clinical remission at the time of enrollment.

Safety, Pharmacokinetic (PK) and Ammonia Sampling

Safety assessments including vital signs, electrocardiograms (ECGs), hematologic and chemistry evaluations were collected. In addition to a safety assessments, patients underwent 12-hour blood sampling for venous ammonia and levels of plasma and urine metabolites including PBA, phenylacetate (PAA), and phenylacetylglutamine (PAGN) on Days 7 (last day of 6 mL BID dosing) and 28 (last day of 9 mL BID dosing), at the following time points: 0 (pre-first, fasting daily dose of GPB), 2, 4, 8 (approximately 2 hours before the second daily dose of GPB), and 12 hours post-first dose (approximately 2 hours after the second daily dose of GPB). Additional ammonia and PK samples were collected on Days 1, 8, 14, 15, and 21 (at pre-first, fasting daily dose and 4 hours post-first daily dose). Urine was collected for PK analysis on Days 7 (for 24 hours) and 28 (for 72 hours).

The protocol was conducted under a US IND. The protocol was reviewed and approved by the Institutional Review Board or Ethics Committee at each investigative site. These included New York Medical College Office of Research Administration, Valhalla, NY; Methodist Health System IRB, Dallas, TX; Baylor College of Medicine, Houston, TX; UT Southwestern Medical Center Institution, Dallas, TX; Western IRB, Olympia, WA; Cleveland Clinic IRB, Cleveland, OH; and Central Ethics Committee of Ministry of Health of Ukraine, Kiev, Ukraine. A Data and Safety Monitoring Board reviewed all safety information, at the end of the open label part and approved the initiation of the randomized part of the study. The study was listed in clinicaltrials.gov with registration number NCT00999167.

Continuous variables were summarized with means, standard deviations, medians, minimums, and maximums. Categorical variables were summarized by counts and by percentage of patients in corresponding categories.

PK Analyses

Concentrations of PBA, PAA and PAGN in plasma and PAA and PAGN in urine were measured by Quest Pharma Services (QPS, LLC, Newark, DE) using liquid chromatography-tandem mass spectrometry.[8–10] Plasma PK parameters calculated for PBA, PAA, and PAGN included the following: maximum observed plasma concentration (C_{max}), minimum observed plasma concentration (C_{min}), and areas under the plasma concentration-time curve from time 0 to 8 (AUC_{0-8h}) calculated using linear trapezoidal rule. Urine was collected on Days 7 and 28 and urine metabolite concentrations measured during the 0–12- and 12–24-hour collection intervals on Days 7 and 28.

Results

Patient Demographics and Disposition

Ten Child Pugh B and 5 Child Pugh C patients (10 males and 5 females) with a mean (SD) age of 52.2 (5.24) years and model for end-stage liver disease (MELD) score of 11.5 (3.66) were enrolled. All 15 patients (100%) completed dosing at 6 mL BID and escalated to 9 mL BID, and 8 patients (53%) completed the study. Seven patients withdrew after the dose escalation to 9 mL BID GPB and prior to completing the 4-week treatment period. Four patients withdrew because they met the pre-specified study stopping rules, and 3 patients withdrew for other adverse events (AEs) that did not correspond to stopping rule criteria. Of the 5 patients with Child-Pugh C liver disease, only 1 completed the study, while 7 of 10 patients with Child-Pugh B completed the study (Table 1).

Safety and Tolerability

Eleven patients (73%) experienced a total of 66 treatment-emergent AEs, excluding HE events. The only AEs reported in more than 1 subject, regardless of relationship to study drug, were diarrhea and hypokalemia in 3 patients each, and upper abdominal pain, nausea

Table 1. Demographics and Baseline Characteristics

Baseline Characteristic	Total (N = 15)
Age (years)	
Mean (SD)	52.2 (5.24)
Median	52.0
Min, max	45, 62
Sex, n (%)	
Male	10 (67%)
Female	5 (33%)
Race, n (%)	
White	14 (93%)
Black or African American	1 (7%)
Body mass index (kg/m^2)	
Mean (SD)	29.5 (5.14)
Median	28.2
Min, max	23, 40
Months in current remission	
Mean (SD)	1.1 (0.87)
Median	0.7
Min, max	0, 3
Child-Pugh classification, n (%)	
A	0
B	10 (67%)
C	5 (33%)
Lactulose use at enrollment (mL/day)	
Mean (SD)	78.0 (41.1)
Median	75.0
Min, max	15, 120
MELD score	
Mean (SD)	11.5 (3.66)
Median	12.0
Min, max	6, 18
HE grade, n (%)	
0	13 (93%)
1	1 (7%)
Not done	1
Asterixis grade, n (%)	
0	13 (93%)
1	1 (7%)
Not done	1

Table 2. AEs Occurring ≥2 Patients

System Organ Class Preferred Term, n (%)[a]	Total (N = 15)
Any AE	11 (73%)
Gastrointestinal disorders	9 (60%)
Diarrhea	3 (20%)
Abdominal pain upper	2 (13%)
Nausea	2 (13%)
Metabolism and nutrition disorders	7 (47%)
Hypokalaemia	3 (20%)
Hypoglycaemia	2 (13%)
Hyponatraemia	2 (13%)
Infections and infestations	4 (27%)
Urinary tract infection	2 (13%)
Nervous system disorders	4 (27%)
Headache	2 (13%)
Blood and lymphatic system disorders	2 (13%)
Thrombocytopenia	2 (13%)
Injury, poisoning and procedural complications	2 (13%)
Investigations	2 (13%)
Musculoskeletal and connective tissue disorders	2 (13%)
Psychiatric disorders	2 (13%)
Renal and urinary disorders	2 (13%)
Skin and subcutaneous tissue disorders	2 (13%)

[a]At each level of summation (overall, system organ class, preferred term), patients reporting more than 1 AE are counted only once. HE events are excluded.

hypoglycemia, hyponatremia, urinary tract infection, headache, and thrombocytopenia in 2 patients each. All 3 patients with diarrhea were on lactulose (Table 2).

Five patients (33%) had 7 serious AEs (SAEs) (excluding HE events), 2 of which led to death; 4 patients experienced an HE event. Related AEs reported in more than 1 patient included diarrhea, nausea, and headache in 2 patients each. There were 2 deaths; one due to esophageal variceal hemorrhage and another due to renal failure. In both cases, the immediate cause of death was judged by the investigator to be unrelated to study drug. The death due to renal failure occurred in a patient with hepatocellular carcinoma who had earlier developed hepatic failure, for which the study drug could not be ruled out as a contributing factor.

The 7 SAEs (excluding HE events) included uncontrolled diabetes (manifested by hyperglycemia), psychosis, liver failure, hypoglycemia, postural orthostatic tachycardia syndrome, renal failure, and esophageal variceal hemorrhage. Of the 7 patients who discontinued from the study, 6 (40%) discontinued the study drug due to AEs and 1 patient discontinued after experiencing an HE event. Patients who discontinued study drug were reported to have the following AEs: psychosis, thrombocytopenia, anemia, uncontrolled diabetes, HE, esophageal variceal hemorrhage, hypokalemia, peripheral edema, abdominal pain, nausea and liver failure. Collectively, the AEs reported by the patients in this study are typical of those expected for the study population.

No clinically significant changes from baseline were observed in vital signs, ECG parameters, hematology or coagulation parameters. The mean value for alanine aminotransferase (ALT) increased during the study from 36.3 U/L at baseline to 46.6 U/L on Day 28/early termination. The mean value for aspartate aminotransferase (AST) also increased, from 56.5 U/L at baseline to 75.5 U/L on Day 28/early termination. These increases were primarily driven by 1 patient who had liver failure

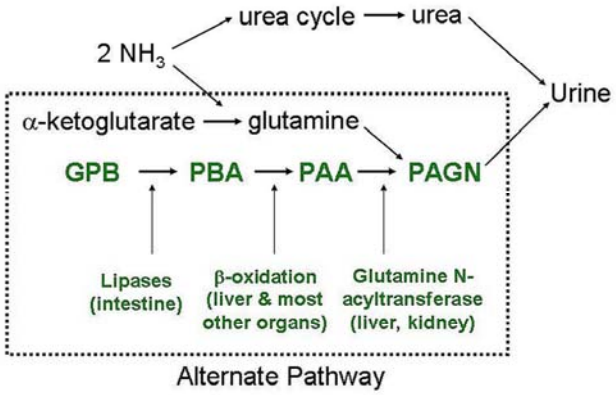

Figure 1. Mechanism of action for GPB. GPB is digested by pancreatic lipases to release PBA which is converted to phenylacetic acid (PAA) by beta-oxidation. PAA is then conjugated with glutamine to form phenylacetyl glutamine (PAGN) which is excreted in the urine, thereby providing an alternate pathway for waste nitrogen excretion.

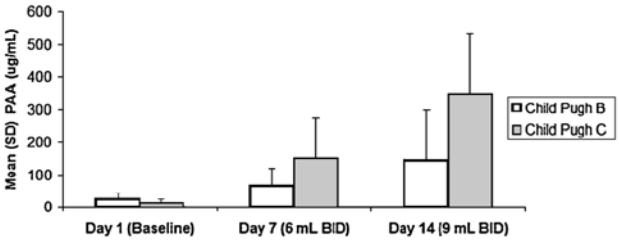

Figure 2. PAA in relation to Child Pugh classification and dose: the panel depicts plasma PAA levels in Child Pugh B and C patients at baseline, Day 7 (n = 10 and n = 5, respectively) and Day 14 (n = 10 and n = 3, respectively). Plasma PAA increased with increasing dose and was higher in patients with more severe liver dysfunction (Child Pugh C vs. Child Pugh B).

with hepatocellular carcinoma due to chronic hepatitis C infection and died due to renal failure. When this patient was excluded, the change in baseline for ALT was to 36.7 U/L and for AST was to 57.6 U/L on Day 28/early termination.

PK Analyses

PBA values for mean C_{min}, C_{max}, and AUC_{0-8h} were all similar during 6 and 9 mL BID dosing. These findings are consistent with prior studies and the short plasma half life of this metabolite (Table 3).[8–10]

Consistent with prior studies in cirrhotic patients, PAA levels increased with repeated GPB dosing and with the GPB dose level and tended to be higher during 9 mL BID than 6 mL BID dosing and were similar on Days 14, 21, and 28, confirming that steady state had been reached by Day 14 (after 7 days at 9 mL BID).[9] The mean C_{min} during 9 mL BID was higher than during 6 mL BID, and the mean C_{max} during 9 mL BID was twice the value of that during 6 mL BID. While the mean AUC_{0-8h} values on Days 7 and 28 were similar, this comparison is confounded by the fact that the highest plasma PAA levels occurred among patients who discontinued the study. PAA levels also were higher in patients with Child-Pugh C than B with $AUC_{0-8\ hr}$ 527 h μg/mL in Child-Pugh B group (N = 10) compared with 1211 h μg/mL in Child-Pugh C (n = 5) and C_{max} of 95 versus 183 μg/mL after 6 mL BID dosing on Day 7.

PAGN mean C_{min}, C_{max}, and AUC_{0-8h} were higher during 9 mL BID compared with 6 mL BID dosing, and the increase in these parameters was roughly proportional to the increase in dose.

PAA levels were generally higher among patients who experienced SAEs and withdrew from the study early

Table 3. Plasma PK Parameters for PBA, PAA and PAGN During GPB Administration of 6 and 9 mL

	PBA		PAA		PAGN	
Parameter	Days 1–7 (6 mL BID)	Days 8–28 (9 mL BID)	Days 1–7 (6 mL BID)	Days 8–28 (9 mL BID)	Days 1–7 (6 mL BID)	Days 8–28 (9 mL BID)
C_{min} (μg/mL)	n = 8	n = 15	n = 8	n = 15	n = 8	n = 15
Mean (SD)	11.3 (12)	8.84 (23.1)	6.33 (4.9)	84.4 (102)	14.5 (6.3)	27.9 (21.9)
Median	7.49	2.18	5.3	44.6	14.9	23.7
Min, max	1.1, 34.8	1.0, 92.1	1.2, 15	1.9, 325	7.5, 24.6	1.2, 65
C_{max} (μg/mL)	n = 8	n = 15	n = 8	n = 15	n = 8	n = 15
Mean (SD)	120 (40.9)	141 (43)	144 (125)	292 (224)	47.6 (14.4)	80.1 (34.7)
Median	115	129	124	219	43.7	63.2
Min, max	51.6, 199	75.8, 231	13.5, 358	57, 655	34.9, 80.9	4.03, 150
AUC_{0-8h} (h · μg/mL)	n = 8	n = 7	n = 8	n = 8	n = 8	n = 8
Mean (SD)	517 (254)	503 (187)	977 (896)	804 (839)	330 (135)	501 (223)
Median	468	511	843	530	316	416
Min, max	87.5, 873	263, 842	43, 2561	187, 2764	192, 620	280, 974

compared with patients who completed the study. Six patients with SAEs had PAA levels ≥300 μg/mL. PAA levels over time for Child-Pugh B and C patients are shown in Figure 2. All but 1 patient with an SAE, who died due to bleeding esophageal varices, had PAA levels that increased substantially after dose escalation from 6.6 g BID (6 mL) to 9.9 g BID (9 mL). These patients also generally had more severe liver disease with higher MELD scores (maximum MELD scores 13–23 vs. 7–18 among patients who completed the study), and 4 out of 5 patients with Child-Pugh C classification at baseline were among this group.

The major urinary metabolite was PAGN. Mean 24-hr excretion of PAGN was 11.7 g while PAA excretion was negligible with a mean of 0.02 g on Day 7. The mean (SD) urinary PAGN output on Day 28 was 1.78 times higher than on Day 7 (11.7 [3.7] g on Day 7 vs. 20.8 [3.7] g on Day 28); that is roughly proportional to the 50% increase in dose, and, on both days, higher during the first 12 hours than during the second 12 hours. Urinary excretion of PAGN continued through at least 72 hours post-dose on Day 28, but approximately 80% of the PAGN excretion occurred during the first 24 hours. The total mean (SD) PAGN excretion over the first 24 hours after the first dose on Day 28 was 20.8 (3.7) g; cumulative excretion from 0 to 72 hours was 25.8 (7.1) g.

Ammonia

Mean (SD) concentration of ammonia at baseline, prior to the first dose of study drug and administration of a meal, was 74.4 (37.5) μmol/L. After the first dose of GPB, blood ammonia concentration decreased to 65.1 (40.7) μmol/L. After 7 days of treatment with 6 mL BID (Day 7) a time point at which data from all 15 patients remaining in the study were available, ammonia concentrations were lowest at pre-dose after overnight fasting and within normal limits (mean [SD] of 47.8 [33.3] μmol/L; ULN = 48 μmol/L). However, ammonia concentrations gradually increased post-prandially during the day reaching the highest concentration at 12 hours post-dose with a mean (SD) of 75.1 (58.5) μmol/L on Day 7. Mean fasting ammonia concentrations were lower at all subsequent time points on GPB relative to fasting ammonia concentrations at baseline. The decreases from fasting ammonia concentrations at baseline were statistically significant at 4 hours post-dose on Day 7 ($P = .047$); at pre-dose fasting ($P = .007$) and 4 hours post-dose ($P = .002$) on Day 8; and at 8 hours ($P = .008$) and 12 hours ($P = .015$) post-dose on Day 28. The largest mean (SD) decrease from baseline was at 4 hours post-dose on Day 8 (−29.7 [36.9] μmol/L). At baseline, 9 patients (69%) had an ammonia value that was above the ULN at the local laboratory. At all subsequent pre-dose time points, ≤50% of patients had ammonia values above the ULN (Figure 3).

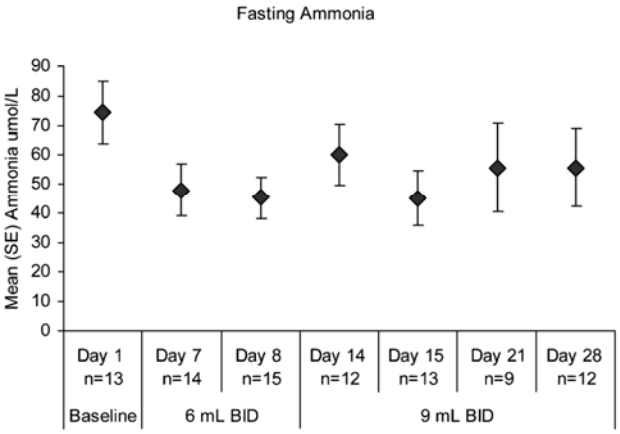

Figure 3. Mean (±SE) fasting ammonia (μmol/L) concentration over time by number of patients (n). As compared with baseline, ammonia concentration was decreased at all subsequent time points.

Discussion

The findings in this pilot study suggest that, similar to findings in patients with UCD, GPB-mediated excretion of waste nitrogen in the form of PAGN appears capable of lowering ammonia concentration in patients with cirrhosis and episodic HE.[8,10] As previously reported in clinically well-controlled UCD patients, ammonia concentrations tended to be lowest after overnight fasting and increased postprandially, thus underscoring the importance of data collection at multiple time points in a controlled environment for assessing ammonia exposure.[8,10] In patients with episodic HE, blood ammonia concentrations decreased after the first dose of GPB despite intake of food, which is typically associated with an increase in ammonia concentration.[8,10,11] At baseline, fasting ammonia concentrations were higher than the average upper limit of normal across sites and were within normal range after 7 days of treatment with GPB. As compared with baseline, ammonia concentration was directionally lower at all time points after starting GPB and fasting ammonia concentration on Day 8, 1 week after starting GPB, was significantly lower than at baseline and within normal limits.

The present data also provide information on dosing. As compared with a GPB dose of 6 mL BID, 9 mL BID provided little additional effect with respect to ammonia control and was less well tolerated, as demonstrated by the frequency of AEs, SAEs, and proportion of patients who tolerated and completed dosing. Phase 1 studies involving intravenous administration of PAA to patients with cancer have demonstrated that PAA levels in the range of 499–1285 μg/mL were associated with reversible toxicity manifested as fatigue, dizziness, dysgeusia, headache, somnolence, lightheadedness, pedal edema, nausea, vomiting, and rash.[12,13] While

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