HEPATOLOGY

HEPATOLOGY, VOL. 67, NO. 3, 2018

Safety, Tolerability, and Pharmacokinetics of L-Ornithine Phenylacetate in Patients with Acute Liver Injury/Failure and Hyperammonemia

R. Todd Stravitz,[1] Michelle Gottfried,[2] Valerie Durkalski,[2] Robert J. Fontana,[3] A. James Hanje,[4] David Koch,[2] Bilal Hameed,[5] Daniel Ganger,[6] Ram M. Subramanian,[7] Stan Bukofzer,[8] William R. Ravis,[9] Kristen Clasen,[2] Averell Sherker,[10] Lanna Little,[11] and William M. Lee [11]; for the Acute Liver Failure Study Group

Cerebral edema remains a significant cause of morbidity and mortality in patients with acute liver failure (ALF) and has been linked to elevated blood ammonia levels. L-ornithine phenylacetate (OPA) may decrease ammonia by promoting its renal excretion as phenylacetylglutamine (PAGN), decreasing the risk of cerebral edema. We evaluated the safety, tolerability, and pharmacokinetics of OPA in patients with ALF and acute liver injury (ALI), including those with renal failure. Forty-seven patients with ALI/ALF and ammonia ≥60 μM were enrolled. Patients received OPA in a dose escalation scheme from 3.3 g every 24 hours to 10 g every 24 hours; 15 patients received 20 g every 24 hours throughout the infusion for up to 120 hours. Plasma phenylacetate (PA) concentrations were uniformly below target (<75 μg/mL) in those receiving 3.3 g every 24 hours (median [interquartile range] 5.0 [5.0] μg/mL), and increased to target levels in all but one who received 20 g every 24 hours (150 [100] μg/mL). Plasma [PAGN] increased, and conversion of PA to PAGN became saturated, with increasing OPA dose. Urinary PAGN clearance and creatinine clearance were linearly related ($r = 0.831$, $P < 0.0001$). Mean ammonia concentrations based on the area under the curve decreased to a greater extent in patients who received 20 g of OPA every 24 hours compared with those who received the maximal dose of 3.3 or 6.7 g every 24 hours ($P = 0.046$ and 0.022, respectively). Of the reported serious adverse events (AEs), which included 11 deaths, none was attributable to study medication. The only nonserious AEs possibly related to study drug were headache and nausea/vomiting. *Conclusion:* OPA was well-tolerated in patients with ALI/ALF, and no safety signals were identified. Target [PA] was achieved at infusion rates of 20 g every 24 hours, leading to ammonia excretion in urine as PAGN in proportion to renal function. Randomized, controlled studies of high-dose OPA are needed to determine its use as an ammonia-scavenging agent in patients with ALF. (HEPATOLOGY 2018;67:1003-1013).

The syndrome of acute liver failure (ALF) evolves rapidly from primary liver injury to secondary multiorgan system failure, and it results in death or the need for liver transplantation in more than half of affected patients.[1] The risk of developing cerebral edema, a systemic complication of ALF which dramatically increases the incidence of poor outcome,[2] is directly proportional to blood ammonia concentrations.[3]

In healthy patients, ammonia produced in the gut is biotransformed into urea and eliminated in urine. In patients with ALF, however, ammonia accumulates in

Abbreviations: AE, adverse event; ALF, acute liver failure; ALI, acute liver injury; AUC, area-under-the-curve; CRRT, continuous renal replacement therapy; ECG, electrocardiogram; GLN, glutamine; HE, hepatic encephalopathy; LOLA, L-ornithine L-aspartate; PA, phenylacetate; PAGN, phenylacetylglutamine; PK, pharmacokinetic(s); ORN, ornithine; OPA, L-ornithine phenylacetate; SRC, Safety Review Committee.

Received June 11, 2017; accepted October 25, 2017.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29621/suppinfo.

Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (grant U-01 58369). Study drug and pharmacokinetic analyses were supplied by Ocera Therapeutics, Inc.

the blood due to deficient activity of hepatic urea cycle enzymes. In the brain, ammonia is taken up by astrocytes and detoxified by glutamine synthetase into glutamine (GLN), an osmotically active molecule.[4] The rapid accumulation of GLN in the brain of patients with ALF is believed to overwhelm compensatory mechanisms that shed intracellular osmolytes, creating an osmotic gradient that results in astrocyte swelling, an increase in brain volume, and intracranial hypertension. Sustained astrocyte swelling can become unresponsive to medical therapy with osmotic agents such as mannitol or hypertonic saline, resulting in uncal herniation and death. Therefore, a strong rationale exists for ammonia-lowering therapies to treat hyperammonemia and prevent cerebral edema in patients with ALF.

Effective ammonia-lowering agents have been developed for infants and children with inborn errors of urea cycle enzymes.[5] Ammonia-lowering agents have received comparatively little study in adults with acute or chronic liver disease. L-ornithine L-aspartate (LOLA) is a compound hypothesized to lower ammonia by providing a precursor for glutamate synthesis (ornithine [ORN]), which serves to bind ammonia to form GLN. Unfortunately, a large, randomized, placebo-controlled study of LOLA in adults with ALF[6] failed to lower blood ammonia levels or improve survival. Jalan and Lee[7] have hypothesized that the lack of efficacy of LOLA in ALF may be due to the deamidation of GLN by glutaminases in the gut and other tissues, thereby liberating ammonia back into the circulation.

Other ammonia-lowering agents have been developed to prevent the deamidation of GLN. Phenylacetate (PA) promotes renal excretion of glutamine and has been used for this purpose in children with urea cycle defects.[8] L-ornithine phenylacetate (OPA), a salt that provides both a substrate for the synthesis of glutamine, ORN, as well as a GLN-scavenging agent, PA, is hypothesized to promote the renal excretion of phenylacetylglutamine (PAGN) (Supporting Figure S1).[9] Studies in rat[10] and pig[11] models of ALF have documented a reduction of blood ammonia during OPA infusion and proved the ability of this compound to act as an effective ammonia scavenger. However, the safety of this compound in humans remains somewhat uncertain considering that astrocyte mitochondria in experimental animals express glutaminases, which have the potential to deamidate GLN and release ammonia, theoretically increasing neurotoxicity.[12]

Previous studies have suggested that OPA is, in fact, safe and well-tolerated in patients with cirrhosis and have demonstrated its ability to promote nitrogen excretion in urine as PAGN.[13,14] The present exploratory study is the first to test the safety and tolerability of escalating doses of OPA in humans with acute liver injury (ALI; no encephalopathy) and ALF, who often have dramatically higher serum ammonia levels than do patients with cirrhosis. Secondary objectives

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.29621

Potential conflict of interest: R. Todd Stravitz has received grants from Ocera. Robert J. Fontana consults for Alynlam and has received grants from Bristol-Myers Squibb, Gilead, and AbbVie. A. James Hanje is on the speakers' bureau for Salix and Intercept. Bilal Hameed has received grants from Intercept, Gilead, and Genfit. Daniel Ganger is on the speakers' bureau for Merck and Gilead. Stan Bukofzer is employed and owns stock in Ocera. William R. Ravis consults for Ocera, Fast-Track, and Lundbeck. William M. Lee consults for Sanofi and Regulus.

ARTICLE INFORMATION:

From the [1]Virginia Commonwealth University, Richmond, VA; [2]Medical University of South Carolina, Charleston, SC; [3]University of Michigan, Ann Arbor, MI; [4]Ohio State University, Columbus, OH; [5]University of California at San Francisco, San Francisco, CA; [6]Northwestern University, Chicago, IL; [7]Emory University, Atlanta, GA; [8]Ocera Therapeutics, Durham, NC; [9]Auburn University, Tuscaloosa, AL; [10]National Institute of Diabetes, Digestive and Kidney Disease, Bethesda, MD; and [11]University of Texas, Southwestern Medical Center, Dallas, TX.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

R. Todd Stravitz, M.D.
Medical Director of Liver Transplantation
Hume-Lee Transplant Center of
Virginia Commonwealth University
P.O. Box 980341
Richmond, VA 23298-0341
richard.stravitz@vcuhealth.org
Tel.: (804) 828-8514

included the evaluation of the pharmacokinetic (PK) and pharmacodynamics of OPA in hyperammonemic patients with ALI/ALF.

Patients and Methods

Since its founding in 1998, more than 3000 adult patients have been enrolled in the ALF Study Group Registry wherein detailed data and daily biosamples are collected over a period of 7 days. All study patients met criteria for ALF or ALI for the Registry, as defined previously.[1,15] Consent was provided by the patient if there were no evident hepatic encephalopathy (ALI), and the patients' legal next of kin, if the patient exhibited signs of encephalopathy (ALF). Separate consents were obtained for the Registry and the OPA clinical trial. The study was approved by each institution's Internal Review Board, conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

The primary objective of the study was to evaluate the safety and tolerability of ascending doses of OPA in patients with ALF and severe ALI with and without renal impairment. Secondary objectives included the evaluation of the PK and pharmacodynamic profile of OPA in patients with ALI/ALF. Exploratory objectives included the evaluation of the effects of OPA on serum ammonia.

STUDY POPULATION

The study was conducted at eight liver transplant centers in the United States that have demonstrated high-volume enrollment in the ALF Study Group Registry. A total of 301 patients were screened to achieve enrollment of 47 patients meeting all criteria (Supporting Figure S2). Inclusion criteria included age 18-65 years, with ALI or ALF as defined, and a plasma ammonia level $\geq$60 μM within 8 hours of the initiation of OPA infusion (defined as Time 0). Exclusion criteria included a history of chronic liver disease, signs of overt uncal herniation or uncontrolled intracranial hypertension, significant gastrointestinal bleeding, hemodynamic instability defined by a mean arterial pressure of <65 mm Hg after volume resuscitation, QT_c interval on baseline electrocardiogram (ECG) of >500 ms, or a history of heart failure, pulmonary hypertension, or concomitant medications known to interfere with renal excretion of PAGN (haloperidol, valproic acid, and others). Patients with ALI/ALF from pregnancy (acute fatty liver of pregnancy and the hemolysis-elevated liver chemistry-low platelet syndrome), acute Wilson disease, malignancy, and shock/ischemia were also excluded from participation. Lactulose and/or rifaximin were not permitted during the study period. Entry criteria were broadened during the study as early safety and tolerability assessments found no safety signals. Initially, enrollment was limited to patients with acetaminophen-induced ALI/ALF because these patients are most likely to develop cerebral edema. Other etiologies were later admitted to the study as long as subjects met the baseline ammonia criterion of $\geq$60μM.

Data on safety and tolerability were collected from all 47 patients. To assess the efficacy of ammonia-lowering, patients were considered evaluable if they received at least 72 hours of OPA infusion, as defined *a priori*. Eleven patients failed to meet the 72-hour infusion criterion and were thus excluded from the ammonia assessment, but they were included in the pharmacokinetic (PK) assessment. Three of the 47 patients had no plasma samples available for determination of PK because they were removed from the study shortly after enrollment (one patient died, one was transplanted, and one withdrew consent/signed out against medical advice).

STUDY DRUG INFUSION SCHEME

OPA infusions were first administered to patients with relatively normal renal function (defined as a baseline serum creatinine $\leq$1.5 mg/dL), and later, after clearance by a Safety Review Committee (SRC), to patients with impaired renal function (defined as a serum creatinine level >1.5 mg/dL). For safety, OPA infusion doses were administered incrementally, starting at 3.3 g every 24 hours as a constant intravenous infusion. Preliminary studies showed that OPA and *N*-acetylcysteine were compatible for infusion through the same intravenous line and were well-tolerated through peripheral or central venous infusion. Three evaluable patients were enrolled at each dose level before beginning enrollment in the next dose level.

The dose escalation scheme is depicted in Figure 1. The beginning of OPA infusion was defined as Time 0. The first patient cohort received OPA 3.3 g every 24 hours for up to 120 hours. After safety and tolerability were assessed by the SRC, the second group received 3.3 g every 24 hours for 12 hours, then increased to 6.7 g every 24 hours for the remainder of the infusion if there were no safety signals as assessed by the site clinical investigator. The third cohort received the initial 3.3 g every 24 hours, the 6.7 g every

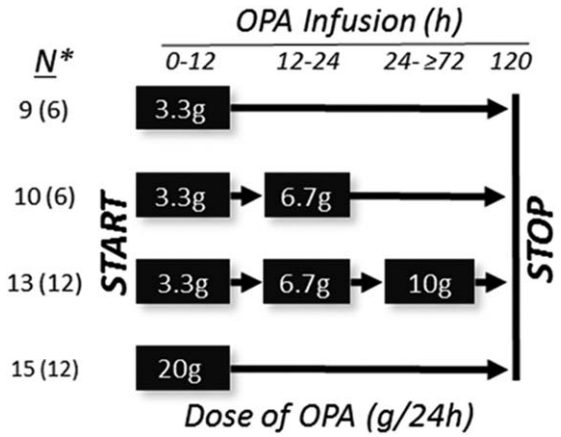

FIG. 1. OPA dose escalation scheme. *Number of patients enrolled into each infusion cohort; data are presented as total number (number of patients evaluable for the ammonia efficacy assessment who received ≥72 hours of OPA infusion).

24 hours dose for the subsequent 12 hours, and then 10 g every 24 hours for the remainder of the infusion, again, assuming there were no safety signals. Finally, because OPA infusions including the dosage of 10 g every 24 hours were deemed safe and well-tolerated by the SRC, a final cohort received a constant infusion of 20 g every 24 hours for up to 120 hours. Some subjects received less than 120-hour infusion because the patient expired, was transplanted, withdrew consent, or improved and was discharged.

CLINICAL EVALUATION

A complete physical examination, laboratory studies including ammonia, plasma, and urine sampling for PK studies, and a safety assessment including ECG for QT_c interval was performed during OPA infusion every 12 hours for the first 3 days and every 24 hours thereafter. Clinical evaluations included neurological assessment, which comprised recording of the hepatic encephalopathy grade according to West Haven Criteria; plasma ammonia, creatinine, and other standard laboratory tests; and urinalysis. All evaluations and samples were also collected at completion of the OPA infusion, and 24 hours after the completion of the infusion.

LABORATORY METHODS

PK studies were performed on plasma from blood samples drawn into Vacutainers containing ethylene diamine tetraacetic acid. Plasma samples for ammonia were collected in heparinized Vacutainer tubes and transported to the laboratory on ice. Samples were run for ammonia according to local protocol. Plasma PA/PAGN/ORN levels were determined using an ultra-performance liquid chromatography method with tandem mass spectrometric detection. The concentration range for all analytes was 5-1000 μg/mL. Urine PAGN levels were determined using an ultra-performance liquid chromatography method with tandem mass spectrometric detection. The concentration range was 300-200,000 μg/mL.

DATA COLLECTION AND MANAGEMENT

The entire study duration for each patient was approximately 32 days; screening assessment, baseline, up to 5 days of treatment, follow up assessment 24 hours after completion of the last infusion, and a post-treatment visit approximately 30 days after treatment day 1 unless terminated early from the trial.

Average plasma concentrations of ORN, PA, and PAGN during the infusions were obtained from the area under the curve (AUC) versus the time from 2-3 days until the end of the infusions, divided by the time period of infusion. Estimates of PAGN renal clearance were calculated by dividing the average daily excretion rate of PAGN by the average PAGN plasma concentration during the same period for each subject. To evaluate drug effects on blood ammonia concentrations, values for the percent decrease from Time 0 were calculated for each sampling time. The average percent decrease in ammonia concentration from 0-72 hours and from 0-120 hours were then determined from the AUC for the percent decrease versus time, divided by the infusion time of OPA.

Data on clinical outcomes were also collected during the study period. All data were collected and reviewed on a secure central server at the Medical University of South Carolina's Data Coordination Unit, which serves as the data coordinating center for the ALF Study Group. Data were assessed through statistical and data management checks to ensure quality and verification.

STATISTICAL ANALYSIS

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Missing values were not replaced or estimated. Descriptive statistics characterized the demographics and other clinical variables. Categorical variables were compared using a

TABLE 1. Baseline Clinical Characteristics of the Entire Study Population According to Renal Function Cohort

Characteristic at Start of OPA Infusion	Normal Renal Function (Creatinine ≤1.5 mg/dL), n = 30	Impaired Renal Function (Creatinine >1.5 mg/dL), n = 17	*P*
ALI, %	46.7	35.3	0.391
HE grade, %			0.340
0/1	56.6	41.2	
2/3	26.7	23.5	
4	16.7	35.3	
OPA ≥72 hours, %	76.7	76.4	0.988
Age, years, median (range)	35.0 (28.0-47.0)	38.0 (30.0-53.0)	0.319
Female sex, %	80.0	41.2	0.007
Acetaminophen, %	83.3	41.2	0.003
Alanine aminotransferase, IU/L, median (range)	4305 (1562-7190)	3695 (892-6025)	0.405
International normalized ratio, median (range)	3.1 (2.5-3.3)	3.3 (2.2-3.7)	0.391
Bilirubin, mg/dL, median (range)	4.0 (2.8-8.0)	7.5 (3.4-17.6)	0.095
Ammonia, μM, median (range)	89.0 (70.0-125.0)	103.0 (73.0-137.0)	0.775
Creatinine, mg/dL, median (range)	0.7 (0.5-0.9)	2.5 (2.0-3.80	<0.0001
CRRT, %	3.3	58.8	<0.0001

chi-squared test or Fisher's exact test (when expected cell counts were <5). Medians were reported with interquartile ranges and compared using a Wilcoxon rank sum test. Plasma concentration results were compared by analysis of variance of log-transformed or rank values. The relationship between baseline creatinine and PK variables were examined with descriptive statistics.

Results

STUDY POPULATION CHARACTERISTICS

Forty-seven patients with ALI/ALF were enrolled into the study, 36 of whom were predefined as evaluable (OPA infusion ≥72 hours) for measurement of ammonia-lowering efficacy (Table 1). The normal renal function cohort (serum creatinine level at screening of ≤1.5 mg/dL [n = 30]) differed significantly from the impaired renal function cohort (creatinine level >1.5 mg/dL [n = 17]) by percent female sex (80 versus 41%, respectively), the proportion with acetaminophen overdose as the etiology (83 versus 41%), and by definition, median serum creatinine (0.7 versus 2.5 mg/dL) and percent on continuous renal replacement therapy (CRRT; 3.3 versus 59%, respectively).

PLASMA CONCENTRATIONS OF STUDY DRUG COMPONENTS AND PRODUCT AT STEADY-STATE

PK assessments included data from 44 of the 47 enrollments. Some of the 8 patients who received the lowest dose of OPA (3.3 g every 24 hours) had undetectably low plasma ORN, PA, and/or PAGN, and were thus omitted from the analyses as shown in Figure 2, which depicts plasma concentrations at steady-state according to maximal infusion rate of OPA. Plasma (ORN) at steady-state (Fig. 2A) was neither related to the infusion rate of OPA nor affected by renal impairment. There was no relationship of plasma ORN to serum creatinine in patients who received the OPA infusion rates of 6.7, 10, or 20 g every 24 hours ($P = 0.76$, 0.52, and 0.79, respectively [data not shown]). In contrast, mean plasma PA (Fig. 2B) increased with the infusion rate of OPA but was not significantly different in patients with normal and impaired renal function. There was no relationship between PA and creatinine concentrations in patients who received the infusion rates of 6.7, 10, and 20 g every 24 hours ($P = 0.10$, 0.38, and 0.71, respectively [data not shown]). Plasma [PAGN] increased not only with increasing OPA infusion rate but also in patients with renal impairment compared with those with normal renal function (Fig. 2C).

EXCRETION OF PAGN

Figure 3 depicts the relationship of plasma PAGN and urinary PAGN excretion to renal function at baseline. As shown in Figure 3A, plasma PAGN increased as a function of baseline creatinine for the infusion rates of 6.7 g ($P = 0.015$), 10 g ($P = 0.003$), and 20 g ($P < 0.001$) every 24 hours. Moreover, renal PAGN clearance was linearly related to creatinine clearance with a slope of 1.18 ($r = 0.831$; $P < 0.0001$ [Fig. 3B]). Renal clearance of PAGN was inversely related to plasma PAGN (Fig. 3C), consistent with renal tubular secretion. The inverse relationship was stronger

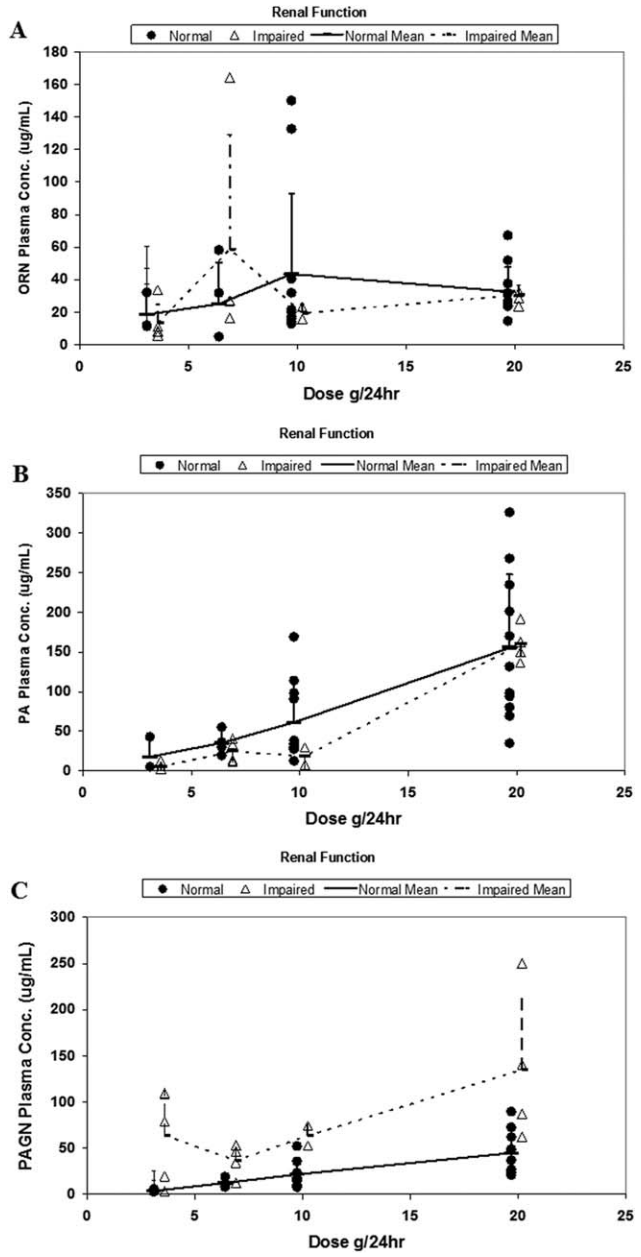

FIG. 2. Plasma concentrations of ORN (A), PA (B), and PAGN (C) according to maximum infusion rate of OPA in normal and renal-impaired subgroups. Data are shown for patients with normal and impaired renal function as defined in Patients and Methods.

in patients with normal renal function ($P < 0.001$) than with impaired renal function ($P = 0.002$).

Eleven patients were treated with CRRT during OPA infusion. Samples of dialysate were also analyzed for PAGN. PAGN concentrations in dialysate were below the limit of detection in all samples.

EFFECTS OF OPA ON SERUM AMMONIA CONCENTRATIONS

The current phase 2a study was not designed to assess the efficacy of OPA in ammonia-lowering nor improvement in grade of HE. Nevertheless, detailed information on ammonia concentrations and HE grade was collected during the study period and follow-up. Ammonia concentrations varied greatly between patients as well as within patients over time. In five study patients, all of whom died, ammonia increased inexorably during OPA infusion. Three of these five patients received the OPA dosage of 3.3 g every 24 hours and died of cerebral edema (n = 1) or multiorgan system failure (n = 2). The other two patients received 10 and 20 g every 24 hours and died of multiorgan system failure (n = 1) and septic shock (n = 1). Although two of the five patients received OPA for ≥72 hours and were considered evaluable in the ammonia-lowering efficacy study, they were hemodynamically unstable and pre-terminal. Thus, the exploratory analysis of OPA on ammonia-lowering was based upon 34 patients, the 36 who received study drug for ≥72 hours excluding the two evaluable but preterminal patients with hemodynamic collapse, a condition which could not support effective ammonia-lowering by OPA.

The distribution of hepatic encephalopathy grades (by West Haven criteria[16]) and respective serum ammonia for each grade at Time 0 is shown in Figure 4. Of all 47 patients in the cohort, 16 had no HE (ALI), whereas grade 1, 2, 3, and 4 HE was recorded in eight, eight, four, and 11 patients, respectively. The patients with HE grades 0-3 all had elevated blood ammonia levels with medians between 84 and 93 μM, whereas the patients with grade 4 coma had significantly higher median ammonia than lower grades (137 μM). Figure 5 depicts the average percent lowering of plasma ammonia based on AUC over two time periods, 0-72 hours (n = 34 patients) and 0-120 hours (n = 29 patients). For patients with data available between 0 and 72 hours, the percent decrease in ammonia AUC concentrations in patients receiving 20 g every 24 hours was larger than in patients who received 3.3 or 6.7 g every 24 hours ($P = 0.048$ and 0.030, respectively). For patients with data available between 0 and 120 hours, the percent decrease in ammonia AUC concentrations in those receiving 20 g every 24 hours was significantly higher than those who received 3.3 or 6.7 g every 24 hours ($P = 0.046$ and 0.022, respectively). We performed a two-factor

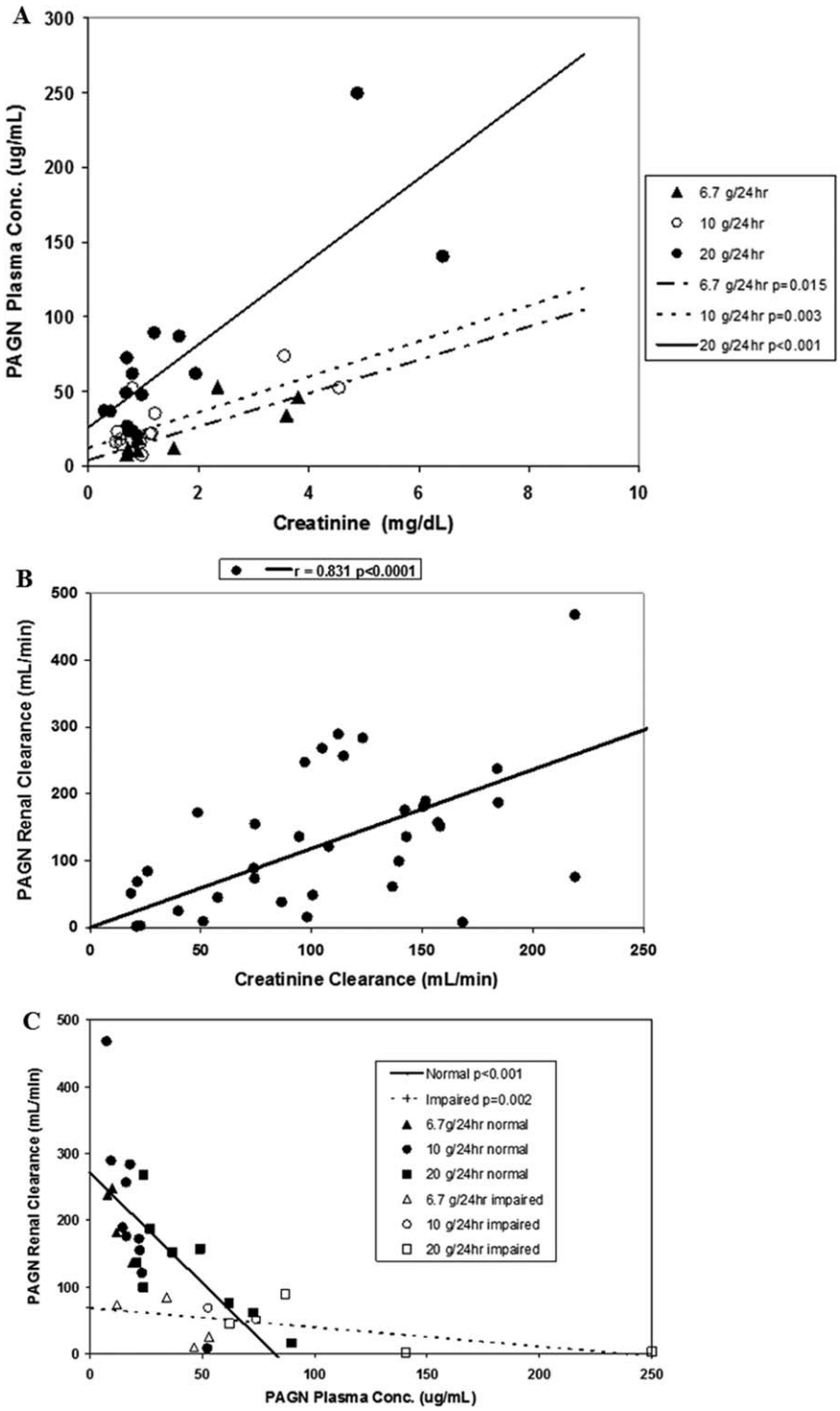

FIG. 3. Concentration of PAGN in plasma and urine according to renal function. (A). Plasma PAGN concentration versus baseline serum creatinine according to maximum infusion rate of OPA. (B). PAGN renal clearance versus creatinine clearance. (C). Relationship of urinary PAGN clearance to plasma PAGN concentration. Data are shown for patients with normal and impaired renal function as defined in Patients and Methods.

analysis examining a possible confounding effect of CRRT on ammonia-lowering by OPA. For the patients with 0-72 hours and 0-120 hours ammonia AUC data, CRRT had no discernable effect on the decrease in ammonia AUC concentrations (P = 0.346 and 0.226, respectively; data not shown). Because most subjects enrolled in the study had no or low-grade (grade 1-2) HE at baseline (n = 32), it was

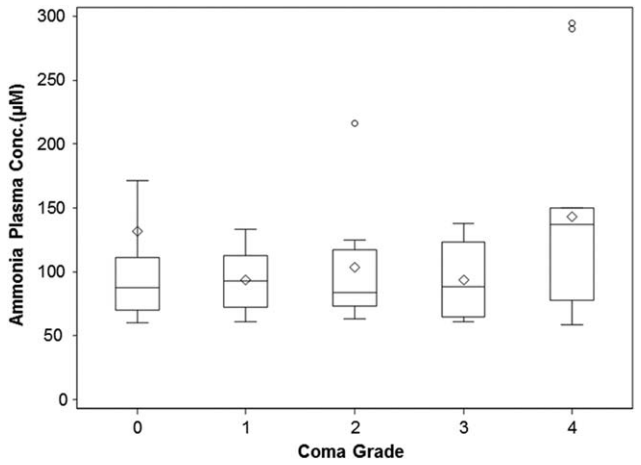

FIG. 4. Plasma ammonia concentrations in the 47-patient study group by hepatic encephalopathy grade at Time 0. The number of patients in each group according to hepatic encephalopathy grade (West Haven Scale) for grade 0 (ALI) and grades 1-4 (ALF) are 16, eight, eight, four, and 11, respectively.

not possible to assess the effects of OPA on HE grade.

SAFETY AND TOLERABILITY OF OPA IN PATIENTS WITH ALI/ALF

Adverse events (AEs) and their relatedness to the study drug are depicted in Table 2 for all 47 patients. All reported AEs classified by MedDRA Term are depicted in Supporting Table S1 according to OPA infusion dose. AEs were expected in this trial because of the severity and global systemic effects of liver failure in patients with the ALF syndrome. Generally, OPA was safe and well-tolerated. Of a total of 107 AEs, 96 (90%) were deemed not related, or unlikely to be related, to the study drug by the site clinical investigator. Similarly, 92 (86%) were deemed non–life-threatening. All of the 26 severe, life-threatening, or fatal AEs were deemed not related, or unlikely to be related, to the study drug. AEs considered possibly related to the study drug by the site clinical investigators included abdominal pain, hypotension, nausea, pyrexia, thrombocytopenia, and vomiting. Six of these cases were deemed mild and two were deemed moderate in severity. AEs considered probably related to the study drug included nausea, vomiting, and headache, two of which were mild and one moderate in severity.

The toxicity of OPA has been ascribed to plasma PA, the levels of which are associated with neurologic AEs when ≥500 μg/mL.[17,18] Neurologic AEs ascribed to PA have included nausea, vomiting, headache, dizziness, lethargy, somnolence, confusion, and dysgeusia. Because the definition of ALF syndrome includes altered sensorium and mentation, lethargy, somnolence and confusion were not specifically captured as AEs. There were seven patients who experienced eight neurologic AEs (nausea, n = 1; headache, n = 4; and vomiting, n = 3). One patient had two reported AEs of vomiting. The median plasma PA in six of seven patients with neurologic AEs (PA was not available for 1 patient) was 16.0 μg/mL (range, 5.0-168.0 μg/mL) compared with 43.7 μg/mL (range, 2.5-490 μg/mL) in patients without neurologic AEs (Wilcoxon $P = 0.388$).

Because biotransformation of PA to PAGN largely occurs in the liver, a plasma PA/PAGN ratio >2.5, reflecting hepatic dysfunction, has been suggested to predict high plasma PA, and an increased likelihood of neurologic AEs in patients with cirrhosis.[8] However, despite plasma PA/PAGN ratios frequently >2.5 in patients with ALI/ALF, the highest plasma AUC PA in any patient regardless of OPA dose or renal dysfunction was 326 μg/mL (Supporting Figure S3), well below the reported neurotoxic threshold.

ECG examinations from 16 patients were electronically flagged by the ECG software as having QT_c

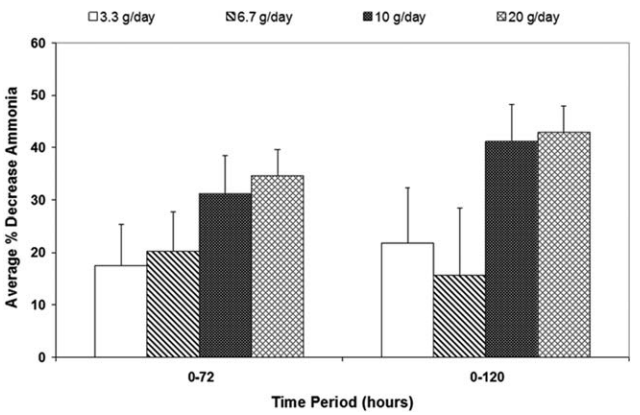

FIG. 5. Decrease in serum ammonia with time of OPA infusion. Data are expressed as a percent change in average serum ammonia concentrations from time 0-72 hours or 0-120 hours according to the maximal dose of OPA infusion (mean ± SEM). *For the AUC 0-72 hours, 20 g every 24 hours versus 3.3 g every 24 hours ($P = 0.048$) and 20 g every 24 hours versus 6.7 g every 24 hours ($P = 0.030$). **For the AUC 0-120 hours, 20 g every 24 hours versus 3.3 g every 24 hours ($P = 0.046$), and 20 g every 24 hours versus 6.7 g every 24 hours ($P = 0.022$).

TABLE 2. Adverse Events and Relatedness to Study Drug

	Relatedness				
Severity	Not Related	Unlikely	Possibly*	Probably†	Total
Total	61 (100.0)	35 (100.0)	8 (100.0)	3 (100.0)	107 (100.0)
Mild	34 (55.7)	16 (45.7)	6 (75.0)	2 (66.6)	58 (54.2)
Moderate	9 (14.7)	11 (31.4)	2 (25.0)	1 (33.3)	23 (21.4)
Severe	5 (8.1)	6 (17.1)	0 (0.0)	0 (0.0)	11 (10.2)
Life-threatening/ Disabling	2 (3.2)	2 (5.7)	0 (0.0)	0 (0.0)	4 (3.7)
Fatal	11 (18.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (10.2)

Data are presented as n (%).
*Events possibly related: abdominal pain, QT_c prolongation >500 ms on ECG, hypotension, nausea, pyrexia, thrombocytopenia (one episode each), and vomiting (two episodes).
†Events probably related: nausea, vomiting, headache.

interval >500 ms during study drug infusion. In all but three cases, the flagged QT_c was found to be inaccurate when reviewed manually by a cardiologist. In these three cases, a more plausible alternative etiology was identified, including severe electrolyte derangement and pericarditis.

Discussion

The incidence of cerebral edema in patients with ALF appears to have decreased in recent years due to improvements in general intensive care and preventative measures.[19,20] Once established, however, cerebral edema remains one of the most highly mortal systemic complications of ALF.[2] Nonspecific medical therapies such as hypertonic saline and mannitol infusions merely provide temporary relief of cerebral edema because they treat the osmotic gradient driving water into astrocytes rather than treating the primary stimulus of the osmotic gradient, hyperammonemia.

In the present study of patients with ALI/ALF, PK data support the two mechanisms of action proposed for the ammonia-scavenging activity of OPA: provision of a substrate for glutamate production (ORN) and an agent to prevent the deamidation of glutamine and promote its renal excretion (PA). Plasma concentrations of ORN were not proportional to the dose of OPA, probably reflecting its rapid conversion to glutamate, suggesting that this step is not rate-determining. The renal clearance of PAGN was strongly correlated with creatinine clearance yet inversely proportional to plasma PAGN, suggesting that the rate-limiting step in ammonia elimination may be renal tubular secretion of PAGN. Although the primary site of synthesis of PAGN from PA and GLN is the liver,[21] we found no relationship between PAGN or the PA/PAGN ratio and the severity of primary liver injury estimated by the international normalized ratio (data not shown). This observation suggests that OPA should be effective as an ammonia scavenger regardless of the severity of liver injury in patients with ALF and is consistent with previous studies reporting similar PAGN renal excretion in healthy volunteers and patients with cirrhosis and normal renal function regardless of Child-Pugh class.[22] However, because the biotransformation of PA to PAGN appears to become saturated at higher doses of OPA, the efficiency of ammonia lowering may be compromised by more severe liver failure.

The ability of CRRT to eliminate PAGN in patients with renal failure remains unproven from this study, since PAGN in all samples of dialysate was below the limit of detection. Although a previous report suggests that PAGN is dialyzable in renal failure patients,[23] the volume of dialysate collected in the present study was inadequate to attempt requantitation on a concentrated sample. However, PK data from one study participant indirectly suggests that CRRT can clear PAGN. The patient, a 33-year-old male with adult Reye syndrome and oliguric renal failure, had the highest blood ammonia of the entire cohort (733 μM on screening and 714 μM at Time 0), and received the highest dose of OPA (20 g every 24 hours) promptly after admission (Supporting Figure S4). At 24 hours of OPA infusion, CRRT was started, and serum ammonia concentrations decreased progressively to 59 and 30 μM at 72 and 96 hours of infusion, respectively. Although PAGN was not detected in the patient's dialysate, plasma PAGN at steady-state reached only 111 μg/mL, a level exceeded by seven other patients in the cohort, four of whom were not receiving CRRT. These observations suggest that CRRT may clear PAGN because plasma PAGN did not rise to very high concentrations despite profound hyperammonemia, and the patient never developed HE or cerebral edema.

A threshold plasma PA to effect ammonia elimination has not been systematically defined. However, based on a study in patients with cirrhosis who received OPA doses of 10 g every 24 hours, the maximal plasma PA of 25 μg/mL was invoked as the reason for a lack of effective ammonia-lowering[14] compared with other studies exploring the administration of glycerol phenylbutyrate (PA 84-292 μg/mL) and sodium phenylacetate (PA >120 μg/mL),[24] both of which were highly effective. We informally chose a plasma PA of ≥75 μg/mL *a priori* as a target level in the present study based on these observations. Thus, many of our patients who received lower doses of OPA did not reach target plasma PA. In fact, only patients who received a dosage of 20 g every 24 hours reliably reached plasma PA of 75 μg/mL; none of those who received 3.3 g every 24 hours met the target, whereas only one patient who received 20 g every 24 hours failed to reach the target. Although previous studies of PA as a chemotherapeutic agent reported a neurotoxic threshold of PA of >500 μg/mL,[17,18] this level was not approached in any patient with ALI/ALF receiving the infusion rate of 20 g every 24 hours regardless of renal failure or PA/PAGN ratio (Supporting Figure S3). Therefore, these data suggest that a dose of 20 g every 24 hours may be optimal for patients with ALI/ALF.

The current study indicates that OPA is both safe and well-tolerated in patients with ALI/ALF. Only a minority of AEs were deemed possibly related to study drug, and all were non–life-threatening. Those AEs probably related to study drug included nausea, vomiting, and headache, all of which were graded as nonserious by the site clinical investigator, and easily could be ascribed to the illness rather than the treatment. These AEs have been described in normal healthy volunteers treated with glycerol phenylbutyrate (a precursor of PA) as a function of increasing PA, but resolved with continued dosing.[8] However, we caution that most of the patients in the current study had altered mentation and may have underreported these potential neurologic AEs. In addition, AEs ascribed to PA in previous studies also included confusion, lethargy, and somnolence,[18] which would have been impossible to accurately distinguish from symptoms of severe liver injury in our patient population.

Although the current phase 2a study was not designed to assess the efficacy of OPA to lower ammonia in patients with ALI/ALF, the data suggest that OPA may be effective as an ammonia scavenger. Indeed, there was evidence of a dose-dependent decrease in average ammonia AUC concentrations between 0-72 hours and 0-120 hours of OPA infusion in patients who received 20 g every 24 hours compared with those who received 3.3 or 6.7 g every 24 hours. CRRT had no discernable effect on ammonia-lowering by OPA, although the number of patients who received CRRT was small. The escalating dose scheme, designed for safety, may explain the delayed effect in ammonia-lowering; the insufficient dose of OPA in patients who received <20 g every 24 hours also played a role. These observations require confirmation with further studies of appropriate design. The effects of OPA on clinical outcome, improvement of HE, or prevention or treatment of cerebral edema could not be assessed and also require further study.

The ammonia-lowering data also suggest that OPA may be futile in a subset of patients who are in the process of dying. Five patients were identified with steady increases in blood ammonia despite OPA infusion, three of whom died of multiorgan system failure, one of cerebral edema, and one of sepsis. Such patients would not be likely to respond to OPA, because intact circulation of OPA, its constituents, and PAGN requires proper delivery to the tissues illustrated in Supporting Figure S1.

In conclusion, the current study suggests that OPA is safe and well-tolerated in patients with ALI/ALF and supports the concept of OPA as a possible ammonia-scavenging agent by the mechanisms proposed previously.[9] Although further studies are required to determine the efficacy of OPA, the preliminary data suggest that 20 g every 24 hours lowers serum ammonia in patients with ALI/ALF.

Acknowledgment: We thank Edward Doo, M.D. (Project Officer, NIDDK) for his tireless support of the ALF Study Group, as well as the following study coordinators: Stephanie S. Taylor, M.S.N., R.N. (Virginia Commonwealth University); Nicole O'Bleness Gray, C.N.P. (Ohio State University); Jeanne Gottstein (Northwestern University); Melanie Crolley, R.N. (Medical University of South Carolina); Rivka S. Elbein, R.N., B.S.N., C.C.R.C. (Emory University); Alina Dobai (University of California, San Francisco); Cassandra S. Coffman, B.S. (University of Michigan); and Deborah Rowan, L.V.N. (University of Texas, Southwestern). We also gratefully acknowledge the contributions of Data Coordinating Center Managers Sarah Williams and Holly Tillman (Medical University of South Carolina), and Safety Review Committee Members Jorge Rakela, M.D. (Medical Safety Monitor and SRC

Chairman; Mayo Clinic, Scottsdale), Jeffrey Browning, M.D. (Internal Medical Monitor; UT Southwestern Medical Center), Constantine J. (Dean) Karvellas, M.D. (University of Alberta, Edmonton, CA), Willis Maddrey, M.D. (UT Southwestern Medical Center), and Ray Chung, M.D. (Massachusetts General Hospital).

REFERENCES

1) Reuben A, Tillman H, Fontana RJ, Davern T, McGuire B, Stravitz RT, et al. Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. Ann Intern Med 2016;164:724-732.
2) Kumar R, Shalimar, Sharma H, Prakash S, Panda SK, Khanal S, et al. Persistent hyperammonemia is associated with complications and poor outcomes in patients with acute liver failure. Clin Gastroenterol Hepatol 2012;10:925-931.
3) Bernal W, Hall C, Karvellas CJ, Auzinger G, Sizer E, Wendon J. Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. HEPATOLOGY 2007;46:1844-1852.
4) Mpabanzi L, Jalan R. Neurological complications of acute liver failure: pathophysiological basis of current management and emerging therapies. Neurochem Int 2012;60:736-742.
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.
6) Acharya SK, Bhatia V, Sreenivas V, Khanal S, Panda SK. Efficacy of L-ornithine L-aspartate in acute liver failure: a double-blind, randomized, placebo-controlled study. Gastroenterology 2009;136:2159-2168.
7) Jalan R, Lee WM. Treatment of hyperammonemia in liver failure: a tale of two enzymes. Gastroenterology 2009;136:2048-2051.
8) Mokhtarani M, Diaz GA, Rhead W, Berry SA, Lichter-Konecki U, Feigenbaum A, et al. Elevated phenylacetic acid levels do not correlate with adverse events in patients with urea cycle disorders or hepatic encephalopathy and can be predicted based on the plasma PAA to PAGN ratio. Mol Genet Metab 2013;110:446-453.
9) Jalan R, Wright G, Davies NA, Hodges SJ. L-Ornithine phenylacetate (OP): a novel treatment for hyperammonemia and hepatic encephalopathy. Med Hypotheses 2007;69:1064-1069.
10) Davies NA, Wright G, Ytrebo LM, Stadlbauer V, Fuskevag OM, Zwingmann C, et al. L-ornithine and phenylacetate synergistically produce sustained reduction in ammonia and brain water in cirrhotic rats. HEPATOLOGY 2009;50:155-164.
11) Ytrebo LM, Kristiansen RG, Maehre H, Fuskevag OM, Kalstad T, Revhaug A, et al. L-ornithine phenylacetate attenuates increased arterial and extracellular brain ammonia and prevents intracranial hypertension in pigs with acute liver failure. HEPATOLOGY 2009;50:165-174.
12) Rama Rao KV, Norenberg MD. Glutamine in the pathogenesis of hepatic encephalopathy: the trojan horse hypothesis revisited. Neurochem Res 2014;39:593-598.
13) Ventura-Cots M, Arranz JA, Simon-Talero M, Torrens M, Blanco A, Riudor E, et al. Safety of ornithine phenylacetate in cirrhotic decompensated patients: an open-label, dose-escalating, single-cohort study. J Clin Gastroenterol 2013;47:881-887.
14) Ventura-Cots M, Concepcion M, Arranz JA, Simon-Talero M, Torrens M, Blanco-Grau A, et al. Impact of ornithine phenylacetate (OCR-002) in lowering plasma ammonia after upper gastrointestinal bleeding in cirrhotic patients. Therap Adv Gastroenterol 2016;9:823-835.
15) Koch DG, Speiser JL, Durkalski V, Fontana RJ, Davern T, McGuire B, et al. The natural history of severe acute liver injury. Am J Gastroenterol 2017;112:1389-1396.
16) Conn HO, Leevy CM, Vlahcevic ZR, Rodgers JB, Maddrey WC, Seeff L, et al. Comparison of lactulose and neomycin in the treatment of chronic portal-systemic encephalopathy. A double blind controlled trial. Gastroenterology 1977;72:573-583.
17) Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor AO, Tompkins AC, et al. A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res 1994;54:1690-1694.
18) Thibault A, Samid D, Cooper MR, Figg WD, Tompkins AC, Patronas N, et al. Phase I study of phenylacetate administered twice daily to patients with cancer. Cancer 1995;75:2932-2938.
19) Bernal W, Hyyrylainen A, Gera A, Audimoolam VK, McPhail MJ, Auzinger G, et al. Lessons from look-back in acute liver failure? A single centre experience of 3300 patients. J Hepatol 2013;59:74-80.
20) Murphy N, Auzinger G, Bernel W, Wendon J. The effect of hypertonic sodium chloride on intracranial pressure in patients with acute liver failure. HEPATOLOGY 2004;39:464-470.
21) Moldave K, Meister A. Enzymic acylation of glutamine by phenylacetic acid. Biochim Biophys Acta 1957;24:654-655.
22) McGuire BM, Zupanets IA, Lowe ME, Xiao X, Syplyviy VA, Monteleone J, et al. Pharmacology and safety of glycerol phenylbutyrate in healthy adults and adults with cirrhosis. HEPATOLOGY 2010;51:2077-2085.
23) Zimmerman L, Jornvall H, Bergstrom J. Phenylacetylglutamine and hippuric acid in uremic and healthy subjects. Nephron 1990; 55:265-271.
24) Ghabril M, Zupanets IA, Vierling J, Mantry P, Rockey D, Wolf D, et al. Glycerol phenylbutyrate in patients with cirrhosis and episodic hepatic encephalopathy: a pilot study of safety and effect on venous ammonia concentration. Clin Pharmacol Drug Dev 2013;2:278-284.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29621/suppinfo.