

# Impact of ornithine phenylacetate (OCR-002) in lowering plasma ammonia after upper gastrointestinal bleeding in cirrhotic patients

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## Abstract

**Background:** Ornithine phenylacetate (OP) has been proven effective in lowering ammonia plasma levels in animals, and to be well tolerated in cirrhotic patients. A trial to assess OP efficacy in lowering plasma ammonia levels *versus* placebo in cirrhotic patients after an upper gastrointestinal bleeding was performed. The primary outcome was a decrease in venous plasma ammonia at 24 hours.

**Methods:** A total of 38 consecutive cirrhotic patients were enrolled within 24 hours of an upper gastrointestinal bleed. Patients were randomized (1:1) to receive OP (10 g/day) or glucosaline for 5 days.

**Results:** The primary outcome was not achieved. A progressive decrease in ammonia was observed in both groups, being slightly greater in the OP group, with significant differences only at 120 hours. The subanalysis according to Child–Pugh score showed a statistically significant ammonia decrease in Child–Pugh C-treated patients at 36 hours, as well as in the time-normalized area under the curve (TN-AUC) 0–120 hours in the OP group [40.16 µmol/l (37.7–42.6); median (interquartile range) (IQR)] *versus* placebo group [65.5 µmol/l (54–126);  $p = 0.036$ ]. A decrease in plasma glutamine levels was observed in the treated group compared with the placebo group, and was associated with the appearance of phenylacetylglutamine in urine. Adverse-event frequency was similar in both groups. No differences in hepatic encephalopathy incidence were observed.

**Conclusions:** OP failed to significantly decrease plasma ammonia at the given doses (10 g/day). Higher doses of OP might be required in Child–Pugh A and B patients. OP appeared well tolerated.

**Keywords:** ammonia, cirrhosis, hepatic encephalopathy, ornithine phenylacetate, upper gastrointestinal bleeding

## Introduction

Hepatic encephalopathy (HE) is a major complication of cirrhosis associated with high mortality and poor quality of life [Ferenci *et al.* 2002]; it also carries a high economic and social burden [Patel *et al.* 2012; Stepanova *et al.* 2012]. Multiple risk factors have been classically associated with the development of HE, including upper gastrointestinal bleeding (UGIB). UGIB induces a status

of hyperammonemia through the intraluminal digestion of blood-nitrogenous compounds by colonic bacteria. In addition, the catabolic status caused by gastrointestinal bleeding provokes an increase in ammoniogenesis in different tissues [Olde Damink *et al.* 2003]. Ammonia and its transformation to glutamine in the astrocyte appear to be a key factor in HE development and pathogenesis [Albrecht and Norenberg, 2006;

*Ther Adv Gastroenterol*

2016, Vol. 9(6) 823–835

DOI: 10.1177/  
1756283X16658252

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Butterworth, 2003; Shawcross *et al.* 2005]. Current strategies for the treatment of HE have been focused on lowering ammonia production through the administration of nonabsorbable disaccharides (NADs) or antibiotics. These therapies have been proved to be effective in HE primary and secondary prophylaxis [Agrawal *et al.* 2012; Bass *et al.* 2010; Sharma *et al.* 2012], but no effects on overt HE episodes or in survival have been shown [Als-Nielsen *et al.* 2004; Bass *et al.* 2010], except for rifaximin, with promising results in overt HE, but with a short period of follow up [Sharma *et al.* 2013]. Other strategies are based on promoting ammonia elimination; glycerol phenylbutyrate has proved its efficacy in secondary prophylaxis, but it has not been tested in cirrhotic-decompensated patients [Rockey *et al.* 2014]. Ornithine phenylacetate (OP) is a new drug proposed as an ammonia scavenger. This therapy is based on the capacity of ornithine to stimulate the activity of glutamine synthetase, hence incorporating ammonia into a 'nontoxic' molecule. The newly formed glutamine will combine with phenylacetate (PA), allowing the elimination of ammonia in the urine by its conversion into phenylacetylglutamine (PAGN). This strategy prevents the degradation of glutamine by intestinal glutaminase and avoids new formation of ammonia [Jalan *et al.* 2007]. OP has been proven to be effective in animal models [Oria *et al.* 2012] and well tolerated in cirrhotic-decompensated patients [Ventura-Cots *et al.* 2013]. OP has been evaluated in a phase I study conducted in healthy volunteers, as well as in a phase IIa study in patients with stable hepatic cirrhosis. The results confirmed that OP was well tolerated and had linear pharmacokinetics at doses up to 10 g infused over 4–24 hours [Hassanein *et al.* 2012]. Although our prior data supported the concept that this molecule might be effective in lowering ammonia [Ventura-Cots *et al.* 2013], no randomized clinical trial compared with placebo had been performed. The goal of the current study was to confirm the efficacy of OP in lowering plasma ammonia in patients with cirrhosis and UGIB, a relevant clinical scenario associated with high mortality and important complications [de Franchis, 2010; Garcia-Tsao and Bosch, 2010].

## Methods

The study was a randomized, double-blind, placebo-controlled, multicenter study comparing the efficacy of OP *versus* placebo in cirrhotic patients with UGIB. Thirty-eight consecutive patients recruited from September 2012 to August 2014

admitted to the Hospital Vall d'Hebron and Hospital de la Santa Creu i Sant Pau (both in Barcelona) were included. A block size of 10 was used to randomize patients; randomization was also stratified by the centers.

The Institutional Review Boards at the study site and the Spanish Agency of Medications and Sanitary Products approved the study according to the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from subjects prior to enrolment. This study is a registered trial [ClinicalTrials.gov identifier: NCT01434108; EudraCT number: 2009-017819-16].

## Patient selection

Subjects were eligible for inclusion if they met the following criteria: age between 18 and 85 years, had a diagnosis of cirrhosis as defined by clinical, laboratory or radiological findings and experienced a UGIB that was active within 24 hours prior to inclusion.

Subjects were excluded from enrolment if they exhibited one of the following criteria: need of mechanical ventilation, terminal illness, infection by human immunodeficiency virus, neurological comorbidities that impaired mental status, suspected hypersensitivity or allergic reaction to the drug components, electrocardiogram with QTcF > 500ms (Bazzet formula), creatinine > 1.5 mg/dl or need for hemodialysis. Additional causes of exclusion were the use of the following drugs: penicillin, probenecid, haloperidol, valproic acid, and systemic corticosteroids (because of their potential interaction with the study drug) and rifaximin (per protocol). In premenopausal women, a negative result in pregnancy test was required and breastfeeding was forbidden.

## Study protocol

Patients were admitted to a semi-intensive care unit (Bleeding Unit) for at least 48 hours, until clinical stability was established. After this period of time, patients were transferred to a general-medicine ward. The standard protocol for the management of UGIB included: intravenous fluids (glucose and saline), perfusion of somatostatin (6 mg/24 hours), use of broad-spectrum antibiotics (ceftriaxone 1 g/daily, intravenously), omeprazole (20 mg/12 hours), nasogastric aspiration of blood and lactulose (initially as enema/12

hours; later orally, adjusted to 2–3 bowel movements/day). The use of rifaximin was prohibited as per protocol. Upper gastrointestinal endoscopy was performed during the first 24 hours and therapy was administered according to findings.

Study medication was prepared in 200 ml empty flasks (10 g/50 ml vials of OP diluted in 150 ml vials of sterile water for injection, or placebo, 200 ml glucose–saline solution) by the pharmacy department and blindly delivered to the study investigators. Medication was administered as an intravenous–continuous infusion, at 0.416 g/h for 5 days (10 g/day), provided the patients did not develop a stopping or withdrawal criteria. In addition, 1 day was required to carry out a clinical and biochemical assessment, and finally, a follow up at 28 days was performed.

Blood samples (10 ml) were collected every 12 hours during the first 48 hours and daily thereafter (up to day 6). A venous access was chosen to avoid discomfort to patients and because venous ammonia shows a good correlation with arterial ammonia [Ong *et al.* 2003]. Urinary samples were collected every 12 hours during the first 48 hours and daily thereafter (up to day 6). Complete urine collection was obtained *via* urinary catheter during the first 48 hours and by supervised 24-hour urine collection after catheter removal.

A follow up at day 28 was performed. Blood samples were centrifuged within 15 minutes of sampling and stored frozen at  $-80^{\circ}\text{C}$ .

### Outcomes

The primary outcome was a decrease in average venous plasma ammonia at 24 hours. Secondary outcomes included: changes in plasma amino acids and ammonia between both groups at any timepoint; pharmacokinetic profiles of PA and ornithine in plasma, and PAGN in urine in order to explore mechanistic processes; HE [presence, duration and severity measured by the West Haven and Clinical Hepatic Encephalopathy Staging Scale (CHESS) scales]; bacterial infections during treatment period; and safety and tolerability of OP.

### Analytical procedures

Ammonia was collected in refrigerated tubs, stored in ice until centrifugation (15 minutes, 3000 rpm,  $4^{\circ}\text{C}$ ), and frozen at  $-80^{\circ}\text{C}$ ; the whole

process was always performed in less than 15 minutes. Ammonia and amino-acid measurements were centralized in hospital Vall d'Hebron and performed in the Laboratory of Metabolic Diseases. Standard laboratory tests included: hemogram, reticulocyte count, prothrombine time and bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, albumin, sodium, potassium, creatinine and calcium plasma concentration.

Ammonia was measured in a Cobas 6000 analyzer (Roche Diagnostics Indianapolis, IN, USA) by standard methodology. Physiologic-free amino acids were analyzed as aminoquinoline derivatives by reverse-phase ultra-performance liquid chromatography (UPLC), Acquity-UPLC (AccQ-Tag chemistry, MassTrak AAA method and instruments, Waters, Milford, MA, US), using a binary solvent gradient at  $42.5^{\circ}\text{C}$  and spectrophotometric detection at 260 nm following manufacturer instructions. Prior to derivatization and chromatography, an internal standard (norvaline) was added and samples were deproteinized by ultrafiltration [Narayan *et al.* 2011]. PA and PAGN were analyzed by liquid chromatography–tandem mass spectrometry according to the protocol set out by Laryea and colleagues [Laryea *et al.* 2010], with some modifications. PA and PAGN were analyzed by liquid chromatography–tandem mass spectrometry. (a) Reagents: deuterium-labeled internal-standard [N-(phenyl- $d_5$ -acetyl)-L-glutamine] ( $d_5$ -PAG) (CDN isotopes, Pointe Claire, QB, Canada). (b) Sample preparation. (1) Standards: the calibration curve was prepared by diluting stock solutions of each standard with MiliQ water to cover a 0–12.92  $\mu\text{mol}$  of PA and 0–32.30  $\mu\text{mol}$  of PAGN concentration range, adding  $d_5$ -PAGN as internal standard (until 3.5  $\mu\text{mol}$  final), in conical plastic centrifuge tubes. (2) Plasma: 100  $\mu\text{l}$  diluted 1:5 plasma was mixed with  $d_5$ -PAGN (as in standards) and was ultrafiltered (10,000g  $\times$  30 minutes). (3) Urine: 100  $\mu\text{l}$  0.22  $\mu\text{mol}$  filtered urine was diluted 1:200 with MiliQ water and mixed with  $d_5$ -PAGN (as in standards). (c) Liquid chromatography–tandem mass spectrometry: instrumentation consisted of an Acquity-UPLC separation module (Waters, Milford, MA, USA) coupled to a Xevo TQ mass-spectrometry system with the Mass Lynx software (Waters, Manchester, UK). The analytical column was an Acquity-UPLC HSS C18 (2.1 mm  $\times$  100 mm). Sample elution was achieved by a 3-minute mobile-phase linear gradient consisting of an initial 40% solvent

A (methanol) and 60% solvent B (1% formic acid in water), changing to 80% solvent A and 20% B from 0–2 minutes, back to 40% solvent A/60% B from 2–2.1 minutes, and equilibrating with these conditions from 2.1–3 minutes. Some 5 µl supernatant samples were injected into the system and the column temperature was maintained at 40°C. Conditions for the mass-spectrometry analysis were: the ionization mode was a negative electrospray (ESI<sup>-</sup>); capillary voltage was 3kV; desolvation gas, heated to 450°C and delivered at a flow rate of 1100 l/h; and cone-gas flow (argon) was 0.15 ml/min. Multiple reaction-monitoring transitions were optimized at the following conditions: dwell time: 0.073 seconds, PAgN: precursor ion 263 (m/z), product ion 145 (m/z), cone voltage 30 V, collision energy 15 eV; PA: precursor ion 135 (m/z), product ion 91 (m/z), cone voltage 20 V, collision energy 10 eV; d5-PAgN: precursor ion 268 (m/z), product ion 145 (m/z), cone voltage 30 V, collision energy 20 eV.

#### Clinical evaluation

Patients were evaluated at the time of initial infusion, every 12 hours during the first 2 days and every day thereafter until day 6; a follow up visit was performed 1 month later. The clinical exam included a 12-lead electrocardiogram, any complications related to cirrhosis, vital signs, and the presence of bacterial infections. The presence of HE was evaluated throughout the study with West-Haven criteria [Conn *et al.* 1977] and the CHES [Ortiz *et al.* 2007].

#### Data analysis

The total sample size of 38 patients (17 patients per group plus 10% of losses) was calculated to detect a difference of  $25 \pm 25$  standard deviation (SD) µmol/l in the mean ammonia average between the two groups at 24 hours (80% power using a one-sided test with  $\alpha = 0.05$ ).

The clinical trial data were independently monitored by the Academic Research Organization of the Vall d'Hebron Institute of Research. A statistical analysis plan was defined between the statistician of the Unit of Statistics and Bioinformatics of Vall d'Hebron Institute of Research and the study investigators before the conclusion of the study. A data-blind review was conducted between the monitors, the statistician and the study investigators before locking the

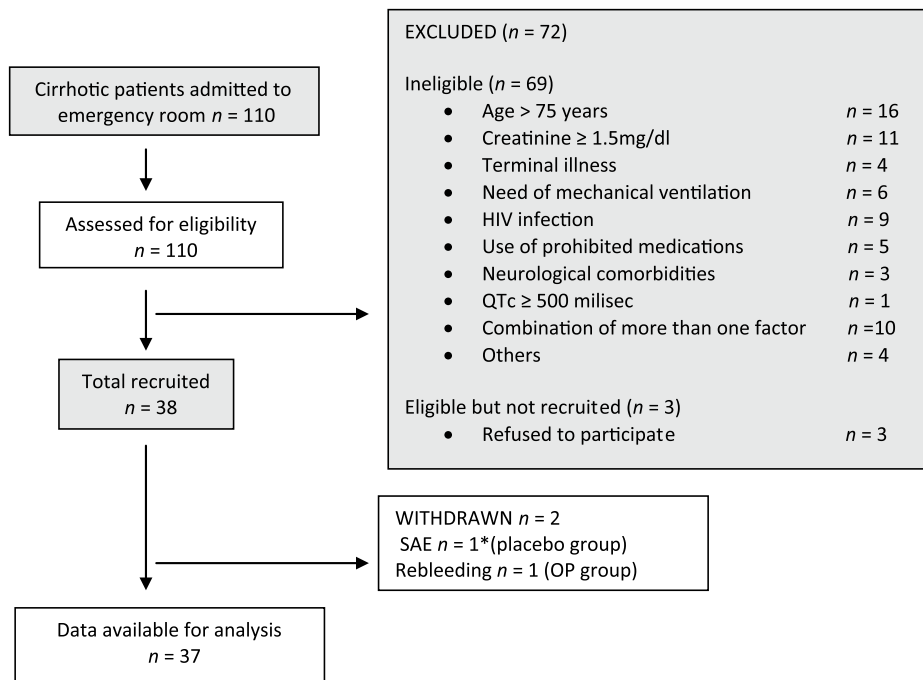
trial database. The statistical analysis was carried out with Stata 13.1. Descriptive statistics were used to summarize the data. Quantitative variables were described as mean  $\pm$  standard deviation for normally distributed variables or median and interquartile range if normality criteria were not met. Normality was assessed by Shapiro-Wilk test and quantitative variables were compared using the one-sided Wilcoxon Rank Sum Test. Percentages were calculated for categorical data. Qualitative variables were described as frequency and percentage, and the one-sided Fisher's exact test was used for calculation. The Wilcoxon Rank Sum Test was used to compare the duration and severity of HE. Ammonia profile and changes were calculated with time-normalized area under the curve through 120 hours (TN-AUC<sub>0–120h</sub>). Subject's regression slopes and group comparisons were made using a one-sided Wilcoxon Rank Sum test.

## Results

#### Characteristics of patients

A total of 110 patients were assessed for eligibility; 72 were excluded for different reasons. The main reason for not participating was age, followed by a creatinine level higher than 1.5 mg/dl. Two patients had to be withdrawn from the study, one of them on day 5 because of a severe adverse event and the other because of a rebleeding during the first 12 hours (Figure 1).

Most of the patients in both groups were males, with a mean age of 55 years. Groups differed in cirrhosis etiology (70% alcohol in the OP group *versus* 44.4% in the placebo group, and 5.5% of hepatitis C virus in the OP *versus* 33.3% in the placebo group). Most of the patients in both groups belonged to Child-Pugh class B, with similar Model for End-stage Liver Disease (MELD) scores. All patients in both groups had presented with previous liver decompensation, being the most common UGIB, followed by ascites. Only one patient in the placebo group and two in the OP group had previously developed overt HE. At inclusion, ascites was the most frequent decompensation event (30% in the OP group *versus* 22.2% in placebo group), followed by HE; no bacterial infections were observed (Table 1). Hematological and biochemical parameters were in accordance of what is expected during a UGIB and no differences between groups were seen at baseline.



**Figure 1.** Flow chart of patient selection.

SAE, severe adverse event; HIV, human immunodeficiency virus; OP, ornithine phenylacetate; QTc, corrected QT interval.  
\*Withdrawal at day 5, data from the first 5 days included in the study.

### Primary outcome

The primary outcome was not achieved. OP failed to significantly decrease plasma ammonia at the given doses. A progressive decrease in ammonia was observed in both groups, being slightly greater in the OP group, with significant differences only at 120 hours (Figure 2). Although the decrease in ammonia at 24 hours in the OP group [ $-20.4 \mu\text{mol/l}$  ( $-39.20$  to  $-2.80$ ); median (IQR)] was almost twice that observed in the placebo group [ $-11.88 \mu\text{mol/l}$  ( $-36.75$  to  $-26.35$ )], differences did not reach statistical significance. The subanalysis by Child–Pugh class showed a statistically significant ammonia decrease in Child C-treated patients at 36 hours ( $p = 0.018$ ), as well as in the TN-AUC<sub>0–120h</sub> in the OP group [ $40.16 \mu\text{mol/l}$  ( $37.7$ – $42.6$ )] versus placebo group [ $65.5 \mu\text{mol/l}$  ( $54$ – $126$ );  $p = 0.024$ ] (Table 2). However, the number of patients in this subgroup analysis was very small.

### Other analytical parameters

A progressive decrease in glycine and glutamine was observed in the treated group as compared with the placebo group, reaching a peak at day 5 in both cases. Differences between both groups started at the 24-hour point and were maintained

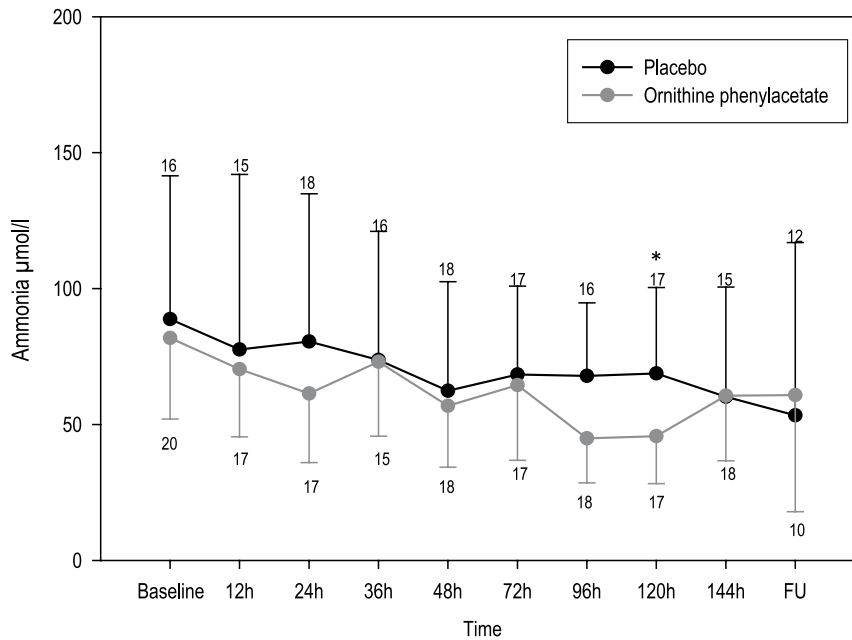
thereafter (Figure 3). Plasma PA levels differed between groups during the entire study (Table 3), the TN-AUC<sub>0–120h</sub> in the placebo group was  $1.6 \mu\text{mol/l}$  ( $0.7$ – $3.3$ ) versus  $111.6 \mu\text{mol/l}$  ( $63.5$ – $148.5$ ) in the OP group ( $p < 0.05$ ). Once the 36-hour dose was reached, PA levels in the OP group remained stable until the end of infusion, never achieving the toxic values that have been reported at a much higher range (approximately  $7000 \mu\text{mol/l}$ ) [Thibault *et al.* 1994]. Urinary levels of PAGN, the main elimination pathway of OP, showed remarkable differences between both groups (Table 3). Total amount of PA excretion accounted for 87% of PA administered as OP at the end of the study.

### Clinical outcome

Per standard of care, all patients underwent an upper gastrointestinal endoscopy during the first 24 hours of admission. The main cause for UGIB in both groups was esophageal variceal bleeding (45% OP versus 83.3% placebo;  $p = 0.1$ ); all of these patients underwent drug treatment and variceal ligation. In the OP group, duodenal or gastric ulcers were frequent etiologies and suitable endoscopic therapies were performed. Three patients in the placebo group and seven in the OP

**Table 1.** Characteristics of patients treated with ornithine phenylacetate and placebo.

	Placebo <i>n</i> = 18	OP <i>n</i> = 20
<b>Demographics</b>		
Age, mean (SD)	56 (11)	55 (10)
Gender (male), <i>n</i> (%)	12 (66%)	14 (70%)
<b>Cirrhosis etiology, <i>n</i> (%)</b>		
Alcohol	8 (44.4%)	14 (70%)
HCV	6 (33.3%)	1 (5%)
HCV & Alcohol	1 (5.6%)	2 (10%)
Other	3 (16.7%)	3 (15%)
<b>Previous decompensation, <i>n</i> (%)</b>		
Ascites	6 (33.3%)	9 (45%)
HE	4 (22.2%)	3 (15%)
SBP	0	2 (10%)
Hepatocarcinoma	1 (5.6%)	1 (5%)
UGIB	6 (33.3%)	8 (40%)
<b>Child–Pugh at inclusion, <i>n</i> (%)</b>		
A	3 (16.7%)	4 (20%)
B	9 (50%)	13 (52.7%)
C	6 (33.3%)	3 (15%)
MELD score at inclusion, mean (SD)	12.2 (3.9)	11.6 (4.4)
<b>Current decompensation, <i>n</i> (%)</b>		
Ascites	4 (22.2%)	6 (30%)
HE	3 (16.3%)	3 (15%)
SBP	0	0
Hepatocarcinoma	1 (5.6%)	1 (5%)
Hepatorenal syndrome	0	0
<b>Analytical parameters, mean (SD)</b>		
Hb (g/dl)	8.7 (1.7)	8.8 (1.7)
Leukocytes x10 <sup>9</sup> /l	7.7 (3.3)	8.5 (3.4)
Platelets x10 <sup>9</sup> /l	98.5 (39.4)	92.1 (44.4)
INR	1.56 (0.26)	1.59 (0.44)
Bilirubin (mg/dl)	2 (1.3)	2.2 (1.33)
ALT (IU/l)	65.8 (91.5)	57.1 (45)
AST (IU/l)	107 (179)	81.9 (57.5)
Creatinine (mg/dl)	0.78 (0.31)	0.88 (0.24)
Albumin (g/dl)	2.9 (0.58)	2.6 (0.54)
Alkaline phosphatase (IU/l)	111.8 (59.6)	114.8 (103)
γ-glutamyl transpeptidase (IU/l)	183.3 (178.6)	158.1 (179)
Na (mEq/l)	139.9 (3.7)	137.3 (6)
Ammonia (μmol/l)	100.3 (43)	94.7 (47)
<b>Vital signs, mean (SD)</b>		
Systolic blood pressure (mmHg)	110 (17)	121 (21)
Dyastolic blood pressure (mmHg)	62.5 (11)	63 (11)
Heart rate (bpm)	79 (13)	80 (15)
Temperature (°C)	36.4 (0.5)	36.4 (0.5)
ECG QTc (millisec)	416 (43)	422 (33)
Red-cell transfusion (units), median (range)	2 (0–4)	2.5 (0–4.5)
OP, ornithine phenylacetate; HCV, hepatitis C virus; HE, hepatic encephalopathy; SBP, spontaneous bacterial peritonitis; SD, standard deviation; UGIB, upper gastrointestinal bleeding; Hb, hemoglobin; INR, international normalized ratio; ALT, Alanine transaminase; AST, Aspartate transaminase; Na, sodium; ECG, electrocardiogram; QTc corrected QT interval. Differences were not significant in any parameter except for HCV etiology; <i>p</i> < 0.05.		



**Figure 2.** Plasma ammonia-concentration evolution during the study period in the ornithine phenylacetate and placebo groups. Data are mean and one-sided standard deviation. Numbers at each timepoint indicate the number of patients with plasma samples analyzed.

FU, follow up.

\* $p < 0.05$ , mean ammonia in treated group at 120 hours was significantly lower than placebo group.

**Table 2.** Ammonia levels expressed as time normalized area under the curve from 0 to 120 hours (TN-AUC<sub>0-120h</sub>) of treated and control patients distributed by Child-Pugh score.

Ammonia µmol/l TN-AUC <sub>0-120h</sub>			
	Median (IQR)		<i>p</i> value
Child-Pugh A	Placebo <i>n</i> = 3 59.69 (56.73–111.80)	OP <i>n</i> = 4 62.29 (57.82–70.47)	0.571
Child-Pugh B	Placebo <i>n</i> = 9 75.79 (59.48–84.94)	OP <i>n</i> = 13 72.74 (46.86–90.26)	0.486
Child-Pugh C	Placebo <i>n</i> = 6 65.50 (53.97–125.76)	OP <i>n</i> = 3 42.57 (37.76–51.09)	0.024

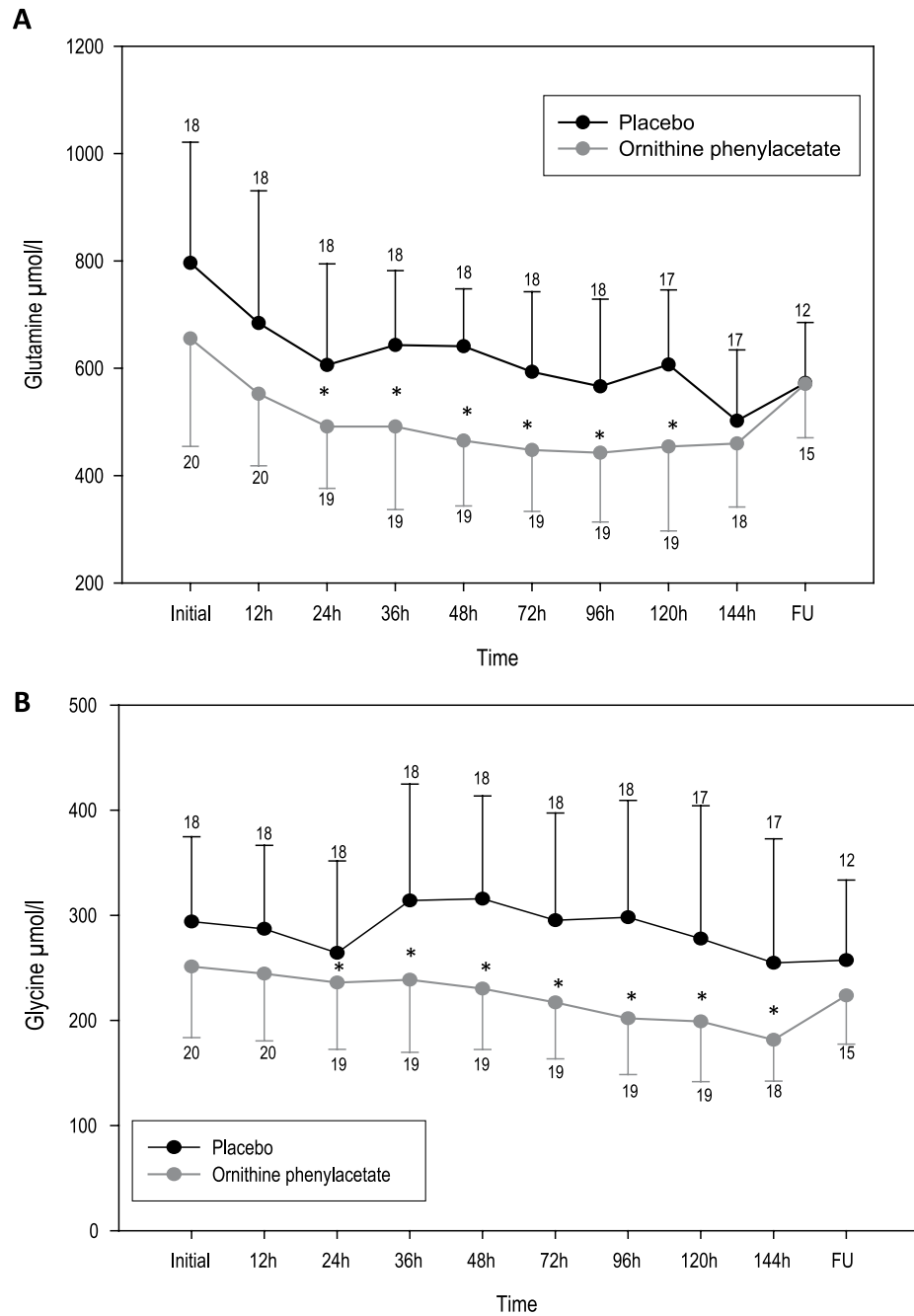
OP; Ornithine phenylacetate, IQR; interquartile range.

group did not require endoscopic treatment. Only one patient in the OP group presented with rebleeding during the first 24 hours and underwent a transjugular intrahepatic portosystemic shunt. Nine patients in the placebo group and 12 in the OP group required blood transfusion; no difference between the total number of blood units transfused and the initial hemoglobin levels were observed between both groups (Table 1). The exposure to blood in the digestive tract was similar for both groups, since the ratio of isoleucine/(leucine plus valine), a good parameter for estimation

of the amount of blood ingested, showed no differences between groups (Supplementary Figure 1) [Dejong *et al.* 1996; Olde Damink *et al.* 1997].

Ascites was the most frequent decompensation event at inclusion: four patients in the placebo group and six in the OP group. Interestingly, three patients in the placebo group and none in the OP group developed ascites during the study.

Three patients in each group exhibited HE at inclusion. The median time of resolution was



**Figure 3.** Glutamine (A) and glycine (B) plasma levels in treated and placebo patients.

FU, follow up.

Data are mean and one-sided standard deviation; numbers at each timepoint indicate the number of patients with plasma samples analyzed.

\* $p < 0.05$ .

24 hours in both groups. One patient in the OP group presented with HE grade III, and two with grade II, while in the placebo group, all patients had HE grade II. During infusion time, two patients in the placebo group (grade II and grade III) and one in the OP group developed HE

(grade I). Lactulose exposure during the study was 90% in the placebo group and 95% in the OP group.

Only one patient in the placebo group presented with a bacterial infection (pneumonia because of



**Table 3.** Levels of phenylacetate in plasma, accumulated phenylacetylglutamine in urine (PAGN<sub>U</sub>), PAGN<sub>U</sub> adjusted to creatinine and percentage of PA eliminated in urine in patients treated with ornithine phenylacetate and placebo.

PA <sub>p</sub> μmol/l Mean (SD)	PAGN <sub>U</sub> accumulated mmol Mean (SD)		PAGN/Creatinine <sub>U</sub> mmol/mol Mean (SD)		% PA eliminated in urine** Molar ratio***
	Placebo   OP	Placebo   OP	Placebo   OP	OP	
Baseline	3.5 (7.7)   2.3 (3.4)				
12h	2.2 (3.3)   89 (52.1)*	1.7 (1.7)   13.5 (9.6)*	325 (287)   2338 (904)*		59
24h	1.3 (2.3)   125.4 (91.9)*	3.1 (2.8)   33.5 (17.2)*	329 (367)   3497 (1084)*		73
36h	0.8 (1)   181.2 (168.4)*	3.8 (3.4)   50.6 (24.1)*	163 (220)   3776 (2723)*		74
48h	1.1 (1.8)   149.2 (96.2)*	4.9 (4.3)   76.6 (32.4)*	178 (287)   3513 (1591)*		84
72h	0.6 (0.8)   140.7 (130.9)*	6 (4.8)   120 (51)*	125 (172)   3571 (952)*		91
96h	0.9 (1.54)   140 (112.2)*	8.3 (7.9)   160 (67.6)*	191 (328)   3764 (1560)*		92
120h	1.1 (1.8)   110.5 (131.4)*	9.5 (9.8)   190 (85)*	178 (232)   3177 (1400)*		87
144h	0.74 (1.1)   0.93 (0.9)	10.6 (11.2)   200 (90)*	130 (175)   284 (672)*		–

OP, ornithine phenylacetate; PA, phenylacetate; PA<sub>p</sub>, phenylacetate in plasma; PAGN<sub>U</sub>, accumulated phenylacetylglutamine in urine; Creatinine<sub>U</sub>, creatinine in urine; SD, standard deviation.  
 \**p* < 0.05, OP group compared with placebo.  
 \*\*In OP group only.  
 \*\*\*Molar ratios were calculated by dividing PAGN<sub>U</sub> accumulated (mmol) by OP administered (mmol), considering that 1 mol of PAGN results from 1 mol of PA (or OP).

bronchial aspiration, secondary to the endoscopic procedure).

#### Safety and tolerability

The incidence of adverse events reported during the study was similar between the OP group (44.72%) and the placebo group (55.28%), as they were the more common adverse events (Table 4). Only one adverse event (nausea) in the OP group was considered related to medication. Ten severe adverse events were reported during the study: four in the placebo group and six in the OP group. Only three of these events were reported during drug infusion: one in the OP group and two in the placebo group (Table 5).

#### Discussion

Based on the results of the present study, OP was not able to obtain a significant decrease in ammonia levels at 24 hours of therapy compared with placebo, and consequently failed to achieve the primary endpoint.

OP has proven safe and well tolerated in cirrhotic-decompensated patients [Ventura-Cots *et al.* 2013] and preclinical studies have shown its potential effect in lowering plasma ammonia

[Oria *et al.* 2012]. Although the trial was not successful in reaching this goal, mean ammonia levels were lower at every timepoint in the OP group, with statistically significant differences at 120 hours. In addition, the TN-AUC<sub>0–120h</sub> was significantly lower in the Child–Pugh C patients of the OP group compared with the placebo group, showing differences of 13.4 μmol/l between groups. Differences of this magnitude been previously described as clinically significant (has been proven effective in decreasing the incidence of HE) in HE secondary-prophylaxis studies [Agrawal *et al.* 2012; Rockey *et al.* 2014]. However, the data obtained in Child–Pugh C patients should not be considered definitive, since the total number of Child–Pugh C patients included was very low.

Similarly to our prior safety and tolerability study [Ventura-Cots *et al.* 2013], only patients with baseline plasma ammonia above the upper normal limit ( $\geq 50$  μmol/l) seem to obtain benefit from ammonia-scavenging drugs. It is important to consider the great individual variability in ammonia levels, not only in the OP group, but also in the placebo group. This variability has been previously described in healthy subjects, patients with urea-cycle disorders and patients with hepatic disease [Mokhtarani *et al.* 2013],

**Table 4.** Most frequent adverse events and severe adverse events in the treated (ornithine phenylacetate) and placebo group during treatment infusion (5 first days) and follow-up period (FU, 28 days).

	Placebo <i>n</i> = 18 patients, <i>n</i> (%) infusion/FU	OP <i>n</i> = 20 patients, <i>n</i> (%) infusion/FU
<b>Organ system preferred term,</b> AE reported in at least in 5% patients		
Investigators		
Hypokalemia	1 (5.5)   1/0	9 (45)   7/2
Hyponatremia	3 (16.7)   3/0	2 (10)   2/0
Alkaline phosphatase increased	0	2 (10)   0/2
Gastrointestinal disorders		
Abdominal pain	3 (16.7)   2/1	1 (5)   0/1
Diarrhoea	2 (11.1)   1/1	1 (5)   0/1
Nausea	0	2 (10)   2/0
Metabolism and nutrition disorders		
Hyperglycemia	5 (27.8)   5/0	8 (40)   7/1
Asthenia	3 (16.7)   0/3	0
Hypocalcemia	2 (11.1)   2/0	0
Blood and lymphatic system disorders		
Leukopenia	2 (11.1)   1/1	1 (5)   1/0
Lymphopenia	3 (16.7)   2/1	0
Neutropenia	1 (5.5)   1/0	3 (15)   3/0
Nervous system disorders		
Dizziness	2 (11.1)   1/1	0
Musculoskeletal disorders		
Cramps	0	2 (10)   0/2
Vascular disorders		
Hypertension	2 (11.1)   2/0	0
General disorders and administration-site conditions		
Pyrexia	6 (33.3)   2/4	3 (15)   1/2
Psychiatric disorders		
Anxiety	0	2 (10)   1/1
Cirrhosis related		
Ascites	2 (11.1)   1/1	0
Edemas	2 (11.1)   1/1	1 (5)   1/0
<b>Any SAE</b>		
Procedural complication		
Hemorrhagic shock post TIPS	0	1 (5)   0/1
Upper-gastrointestinal hemorrhage	1 (5.5)   0/1	2 (10)   0/1
Cirrhosis related		
Hepatic encephalopathy	0	1 (5)   0/1
Nervous system disorders		
Seizures due to cerebellar hemorrhage	1 (5.5)   0/1	0
Subdural hematoma	1 (5.5)   0/1	0
Infections		
Respiratory-tract infection	1 (5.5)   0/1	1 (5)   0/1
Blood and lymphatic-system disorders		
Neutropenia	0	1* (5)   1/0
AE, adverse event; SAE, severe adverse event; TIPS; transjugular porto-systemic shunt; FU, follow up; OP, ornithine phenylacetate. *Causality relationship between study medication and SAE was not established.		

**Table 5.** Total number of adverse events with grading and presentation time in treated (ornithine phenylacetate) and placebo patients.

Severity	Infusion (70)		Follow up (53)		Total (123)	
	Placebo <i>n</i> (%)	OP <i>n</i> (%)	Placebo <i>n</i> (%)	OP <i>n</i> (%)	Placebo <i>n</i> (%)	OP <i>n</i> (%)
	40 (100%)	30 (100%)	28 (100%)	25 (100%)	68 (100%)	55 (100%)
Mild	29 (72.5%)	21 (70%)	22 (78.5%)	12 (48%)	51 (72.8%)	33 (55.9%)
Moderate	9 (22.5%)	8 (26.6%)	4 (14.2%)	8 (32%)	13 (18.5%)	16 (27.1%)
Severe	2 (5%)	1 (3.3%)	2 (7.1%)	5 (20%)	4 (5.7%)	6 (10.1%)

OP, ornithine phenylacetate.

suggesting not only the relevance of sample processing, but the participation of genetic factors, such as the variability in the glutaminase gene [Romero-Gomez *et al.* 2010].

It is important to emphasize that the mean plasma concentration of PA achieved in our treated patients [maximum plasma PA level of 24.7 µg/ml at 36 hours of infusion (181 µmol/l), Table 3] is much lower than the concentrations obtained with similar therapies, such as glycerol phenylbutyrate (Ravicti®) ( $C_{\min}$ : 84.4 µg/ml– $C_{\max}$ : 292 µg/ml) [Ghabril *et al.* 2013; Hyperion Therapeutics, 2014] or sodium phenylacetate (Ammonul®), ( $C_{\min}$ : 120 µg/ml– $C_{\max}$ : 4652 µg/ml) [Food and Drug Administration, 2005]. We hypothesize that higher doses of OP, ranging from 15 to 20 g daily, would obtain higher plasma PA levels and therefore achieve a higher drop in plasma ammonia, to levels that have been historically associated with a low incidence of HE. These higher doses of OP should be probably applied to Child–Pugh A and B patients, since in our subgroup analysis, OP appeared to have a higher effect in Child–Pugh C patients with the current dose of 10 g/day. In addition, data from other studies indicate that Child–Pugh C patients have higher exposure to PA than Child–Pugh A and B patients [Ghabril *et al.* 2013; McGuire *et al.* 2010; Hyperion Therapeutics, 2014]; the efficacy of higher doses of OP should be confirmed in future studies.

The total number of patients included in our study (38 patients) was calculated to detect a difference of  $25 \pm 25$  (SD) µmol/l in the mean ammonia average between the two groups at 24 hours. This calculation was based in the results of our pilot study [Ventura-Cots *et al.* 2013], where the decrease of plasma ammonia during the first

24 hours in the treated group was compared with a historical cohort of 17 patients who exhibited a steady state of plasma ammonia during the first 24 hours; this steady state was not observed in the placebo group of the present study. This fact has been critical to determine the lack of power in the present study to achieve the main objective.

The decrease of plasma glutamine concentration during time, as well as the increased in PAGN detected in urine, confirms the proposed mechanism of action (combining glutamine to PA and enhancing urinary excretion). OP is a new drug that acts by providing an alternate pathway for ammonia removal in the form of urinary PAGN [Jalan *et al.* 2007]. In our study, the recovery of as much as 87% PA administered as urinary PAGN indicates that most of the drug is metabolized and participates in ammonia removal. The plasma amino-acid profile of the OP-group patients also seems to support the existence of other ammonia-removal pathways promoted by OP and previously described in preclinical studies, such as the combination with glycine and its consequent decrease in plasma, followed by their elimination as phenylacetylglutamine in urine [Kristiansen *et al.* 2014]. This hypothesis has not been confirmed in our study, since the urinary determination of phenylacetylglutamine was not carried out.

Our trial had a similar incidence of HE episodes between both groups at baseline, during infusion and during follow up. Taking into account the incidence of HE during cirrhosis decompensation (16–21%) [EASL, 2010] and the sample size of our study ( $n = 38$ ), differences in the incidence of HE were not expected. We did not observe significant differences in baseline and follow-up

ammonia levels in patients with HE, treated with OP or placebo.

The study confirms that OP, at the present dose, is a well-tolerated drug, even in decompensated cirrhotic patients. The incidence of adverse events and severe adverse events was the same in both groups, and it is important to underline that most of the adverse events recorded in both groups developed during the follow-up period, after drug infusion.

OP appears to be a well tolerated and promising drug for the treatment of ammonia-related complications of cirrhosis, even in patients with poor liver function. The current study did not achieve the primary objective, but this was probably due to individual variability, a small number of patients included and mainly, too low a dose of OP administered. On the other hand, the consistent decrease in ammonia levels during therapy in the treated group and the safety profile of OP indicate that with higher doses, especially in Child–Pugh A and B patients, a clinical effect will probably be achieved.

### Funding

This study was funded in part by the Spanish Ministry of Health, grant number, FIS 10/1028. This study was funded in part by the Instituto de Salud Carlos III, grant number, TRA-190. Macarena Simón-Talero is a recipient of a ‘Río Hortega’ fellowship grant from the ISCIII.

### Conflict of Interest statement

Potential competing interests: Juan Córdoba (deceased) received consulting fees from Ocera Therapeutics Inc. The remaining authors declare that they have nothing to disclose.

Study medication was purchased from Ocera Therapeutics Inc., by the promoters of the study.

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