

AMMONIA PRODUCES PATHOLOGICAL CHANGES AND ACTIVATION IN HUMAN HEPATIC STELLATE CELLS AND IS A TARGET FOR THERAPY IN PORTAL HYPERTENSION

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Background: Hepatic stellate cells (HSC) are vital to hepatocellular function and the liver's response to injury. They share a phenotypic homology with astrocytes that are central in the pathogenesis of hepatic encephalopathy, a condition in which hyperammonemia plays a pathogenic role. This study tested the hypothesis that ammonia modulates human HSC activation *in vitro* and *in vivo*, and evaluated whether ammonia lowering, by using L-ornithine phenylacetate (OP), modifies HSC activation *in vivo* and reduces portal pressure in a bile duct ligation (BDL) model.

Methods: Primary human HSCs were isolated and cultured. Proliferation, metabolic activity, morphology, HSC activation, and changes in oxidative status (ROS) and endoplasmic reticulum (ER) were evaluated to identify effects of ammonia challenge (50 μ M, 100 μ M, 300 μ M) over 24-72hrs. Changes in plasma ammonia levels, markers of HSC activation, portal pressure, hepatic eNOS activity and expression of its regulatory proteins were quantified in hyperammonemic BDL animals, and after OP treatment.

Results: Pathophysiological ammonia concentrations caused ~~produced~~ significant and reversible changes in cell proliferation, metabolic activity and activation markers of hHSC *in vitro*. Highly significant alterations in cellular morphology, characterised by cytoplasmic vacuolisation, ER enlargement and ROS production, pro-inflammatory gene expression were observed together with HSC-related activation markers. These changes were confirmed in hyperammonemic BDL animals. Treatment with OP significantly reduced plasma ammonia and portal pressure which was associated with increased eNOS activity, ~~and~~ decreased caveolin-1 and abrogation of HSC activation markers.

Conclusions: The results show, for the first time, that ammonia produces marked morphological, functional and deleterious effects on HSCs *in vitro*. Targeting ammonia with the ammonia-lowering drug OP reduces portal pressure and deactivates hHSC *in vivo*, highlighting the need for evaluating ammonia lowering as a potential therapy in cirrhosis patients with portal hypertension

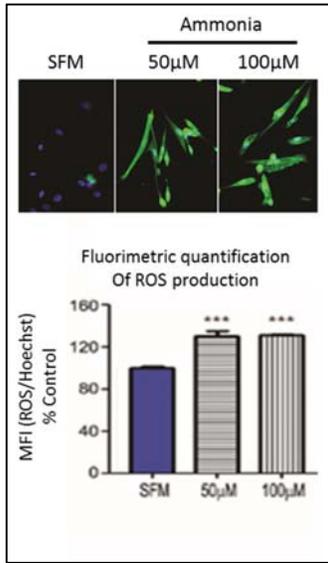


Figure legend: Hyperammonemia induces ROS production in hHSC. The formation of reactive oxygen species (ROS) was measured using Image-IT™ LIVE Green Reactive Oxygen Species Detection Kit (SFM = Serum Free Medium).

Ornithine transcarbamylase gene expression and hepatic urea nitrogen handling are reduced in models of NAFLD and recovers with dietary modulation and reducing bacterial translocation: Rationale for ammonia lowering therapy in NASH patients

(Max 255)

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Background: Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease ranging from steatosis, through non-alcoholic steatohepatitis (NASH) to cirrhosis. In NASH, bacterial translocation and dysfunctional mitochondria may affect the function of the mitochondrial urea cycle enzyme ornithine transcarbamylase (OTC), and result in hyperammonemia, which has been shown to activate hepatic stellate cells *in vivo* and *in vitro*. This may favour the progression of NAFLD. The aims of this study were to determine whether gene and protein expression and function of OTC are altered in animal models of NASH and NAFLD patients. We also determined if this was reversible with recovery of animals by restoring the diet and by reducing bacterial translocation.

Methods: Two animal models of NASH were studied. (a) high-fat, high-cholesterol diet (HFC) for 10 months and then recovery for 2 months (b) methionine-choline deficient diet (MCD) for 4 weeks treated with or without Yaq-001 (Yaqrit Ltd.), a nanoporous carbon which has been shown to reduce bacterial translocation. In humans, we obtained liver biopsies from 16 NAFLD patients during bariatric surgery and measured the OTC gene expression.

Results: In both animal models, gene and protein expression of OTC was reduced significantly. In the HFC rats, reversal of NASH by changing the diet to normal chow restored OTC gene (0.53 (0.41-0.68) vs. 0.32 (0.28-0.37), $P < 0.05$; controls 1.00 (0.85-1.17)) and protein expression (5.33±0.21 vs. 3.06±0.20, $P < 0.01$; controls 9.11±0.68)). In the MCD mice, reduction in bacterial translocation using Yaq-001 prevented development of NASH and restored the OTC gene expression (0.89 (0.13-0.16) vs. 0.35 (0.08-0.09), $P < 0.01$; controls 1.00 (0.12-0.17)) suggesting that inflammation in NASH contributes to OTC gene expression. In the NAFLD patients, those with NASH and fibrosis had significantly lower OTC gene expression than patients with steatosis alone (0.82±0.37 vs. 1.15±0.24, $P < 0.05$).

Conclusion: Experimental and human NASH resulted in a reduction in gene expression of the urea cycle enzyme OTC impairing nitrogen homeostasis. The changes were reversible in the animal models with dietary intervention and also by reducing bacterial translocation. These investigations suggest a link between NASH, reduction in gene expression and function of OTC and bacterial translocation, which can result in hyperammonemia and progression of liver injury and fibrosis supporting a rationale for targeting ammonia and bacterial translocation as a potential treatments for NASH.

Long term oral treatment of ornithine phenylacetate increases lean mass and attenuates brain edema in bile-duct ligated rats.

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Background: Chronic liver disease (cirrhosis; CLD) is characterized by numerous metabolic disturbances which lead to complications that impact the clinical outcome. Among these, loss of muscle, characterized by a deterioration of muscle quantity and quality, leads to a decrease in functional capacity, adversely affecting survival, quality of life and outcome following liver transplantation. Hyperammonemia is central in the development of hepatic encephalopathy, a major complication of cirrhosis. However, it is speculated the toxic effect of ammonia extends beyond the brain, possibly affecting muscle. Therefore, we hypothesized that lowering blood ammonia will attenuate muscle mass loss in cirrhotic rats. Ornithine phenylacetate (OP; OCR-002) was used to lower blood ammonia. **Methods:** We induced CLD in rats following 6-week bile-duct ligation (BDL). Four experimental groups were tested; 1) Sham; 2) BDL; 3) Sham + OP; 4) BDL + OP. One week following BDL, rats were orally administered (gavage) OP (1g/kg) daily for 5 weeks. Body weight, fat and lean mass (EchoMRI), blood ammonia, cerebral edema (specific gravity method), fractional synthesis of protein (FSR) in muscle (with D₂O) and locomotor activity (day/night) were measured. **Results:** At the end of the 6-weeks experiment, BDL rats demonstrated a 4-fold increase in blood ammonia vs Sham-operated controls. This increase was reduced by 40% in OP-treated BDL rats. BDL rats gained less body weight compared to sham-operated controls (body weight of 360.2g ± 13.6 vs 476.8g ± 10.38; p<0.001) which was accompanied with a lower gain of lean mass and a lower muscle FSR. OP-treated BDL rats showed a significant increase in body weight (429.6g ± 117.9; p<0.001 vs BDL) with a significant higher lean mass (303.1g ± 10.7 in BDL+OP vs 264.4g ± 10.5 in BDL, p<0.01). Fat mass remained unchanged between the treated and untreated BDL groups. OP treatment normalized brain water content in BDL rats. In contrast, OP-treatment reduced muscle FSR in SHAM animals, but not in BDL rats. Locomotor activity in BDL rats was reduced compared with sham-operated controls but no significant change was found between BDL+OP and SHAM+OP. **Conclusion:** This is the first study demonstrating the efficient ammonia-lowering effect of an oral formulation of OP. Long-term treatment with OP is a safe, non-antibiotic alternative demonstrating a significant ammonia-lowering effect, as well as a protective effect on the development of brain edema and muscle mass loss in rats with CLD. Whether the effect of OP on muscle mass loss attenuation is a result of lowering blood ammonia or directly improves muscle metabolism remains to be established.

Minimal hepatic encephalopathy leads to hypotension-induced neuronal cell loss in BDL rats

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Background: Hepatic encephalopathy (HE) is a major neuropsychiatric complication caused by liver disease characterized by cognitive and motor dysfunction. The only curative treatment to date remains liver transplantation (LT). Historically, HE has always been considered to be a reversible metabolic disorder and has therefore been expected to completely resolve following LT. However, persisting neurological complications remain a common problem affecting as many as 47% of LT recipients. LT is a major surgical procedure accompanied by intraoperative stress and confounding factors, including blood loss and hypotension. We hypothesize, in the setting of minimal HE (MHE), the compromised brain becomes susceptible to hypotensive insults, resulting in cell injury and death.

Methods: Six-week bile-duct ligated (BDL) rats with MHE and respective controls (SHAM) were used. Blood is withdrawn from the femoral artery (inducing hypovolemia) until a mean arterial pressure of 30 and 60 mmHg (hypotension) and maintained for 120 minutes. Cerebral blood flow (CBF) was assessed by injecting fluorescent microspheres (1×10^6 microspheres/ml) through the brachial artery. Upon sacrifice, brains were extracted for apoptotic analysis (western blot) and neuronal cell count (immunohistochemistry). In a separate group, BDL rats were treated for MHE with ornithine phenylacetate (OP; OCR-002) (1g/kg) for 3 weeks.

Results: Both BDL rats and SHAM-operated controls without hypotension did not display any cell injury or neuronal loss. However, BDL rats following hypotension (30 and 60mmHg) demonstrated a significant decrease in neuronal cell count in the frontal cortex (using NeuN+DAPI and Cresyl Violet) compared to hypotensive SHAM-operated controls. In addition, neuronal loss was associated with an increased in cellular stress protein, hsp32, hsp70 and caspase-3, suggesting apoptotic cell death. CBF decreased in BDL rats compared to SHAM and correlated with degree of hypotension insult. BDL rats treated with OP did not lead to neuronal cell death following hypotension.

Discussion: These findings strongly suggest that cirrhotic patients with MHE are more susceptible to hypotension-induced neuronal cell loss. Moreover, these results suggest a patient with HE (even MHE), with a “frail brain”, will fare worse during liver transplantation and consequently result in poor neurological outcome. Combination of MHE and hypotension may account for the persisting neurological complications observed in a number of cirrhotic patients following LT. Therefore, MHE, i) should not to be ignored and ii) deserves to be treated in order to reduce the risk of neurological complications occurring post-LT.

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PRESENTATION TYPE: Oral or Poster

CURRENT CATEGORY: Portal Hypertension and Other Complications of Cirrhosis

CURRENT DESCRIPTORS: PO4. Encephalopathy and Other Complications

TITLE: Efficacy of Ornithine Phenylacetate (OP) in lowering plasma ammonia after upper gastrointestinal bleeding (UGIB) in cirrhotic patients: a multicenter, randomized, double blind trial

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ABSTRACT BODY:

Abstract Body: OP has been proposed to decrease ammonia in uncontrolled studies. Methods: Thirty-eight consecutive cirrhotic patients, aged between 18-85 years and with a creatinine level lower than 1.5 mg/dL were enrolled within 24 h of an UGIB. Patients were randomized (1:1) to receive OP (10g/24h continuous infusion for 5 days) or placebo, in addition to the usual treatment, and followed up for 28 days. Ammonia and related metabolites were assessed by HPLC and mass spectroscopy. The hypothesis was that OP would produce a 25µmol/L venous ammonia difference in the means between the groups during the first 24h. Secondary outcomes included: plasma ammonia changes at any time point, pharmacokinetic profile, hepatic encephalopathy and clinical outcome, and confirmation of safety and tolerability of OP. Treatment groups were compared using a one-sided exact Wilcoxon rank-sum test. Results: a progressive decrease in ammonia concentration was observed in both groups, being higher in OP group at each time point, without significant differences. Decrease in ammonia in the first 24h in the OP group [-20.40 µmol/L (-39.20, -2.80); median (IQR)] was twice that of the placebo group [-11.88 µmol/L (-36.75, 26.35)], without statistical significance. The subanalysis by Child-Pugh score showed a statistically significant ammonia decrease in Child-Pugh C treated patients at 36 h, as well as in the time normalized area under the curve (TN-AUC) 0-120h in the OP group [40.16 µmol/L (37.7, 42.6)] vs placebo group [65.5 µmol/L (54, 126); p=0.036]. A progressive decrease in plasma glycine and glutamine were observed in the treated group as compared to the placebo group. These differences reached statistical significance after 24 h of treatment and were maintained thereafter. Glutamine decrease associated with the appearance of phenylacetylglutamine in urine: u-PAG accumulated TN-AUC 12-120h placebo [5768 µmol (1769,8808)] vs TN-AUC 12-120h OP [106851µmol (78802, 146666)]; p<0.001]. The frequency of adverse events (AE) reported during the study was similar in both groups, OP=55 (6 SAEs) and placebo=68 (4 SAEs). No differences in hepatic encephalopathy incidence or clinical outcome were observed between the two groups. Conclusions: OP 10g/day decreases plasma ammonia after UGIB in cirrhotic patients, especially in Child-Pugh C patients. Higher doses of OP might be required in Child-Pugh A and B patients to increase ammonia elimination. The higher ammonia decrease in the OP group is related to the elimination of glutamine from plasma and its appearance as PAGN in urine. OP appeared to be safe and was well tolerated.

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(No Image Selected)

Co-Author Disclosure Status

The following authors have completed their AASLD 2015 disclosure:: Meritxell Ventura-Cots: Disclosure completed | German Soriano: No Answer. | Macarena Simon-Talero: No Answer. | Maria Torrens: No Answer. | Albert Blanco: No Answer. | Jose Antonio Arranz: No Answer. | Mar Concepción: No Answer. | Edilmar Alvarado: No Answer. | Cristina Gely: No Answer. | Eva Roman: No Answer. | Joan Genescà: No Answer. | Juan Cordoba: No Answer.

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PRESENTATION TYPE: Oral or Poster

CURRENT CATEGORY: Acute Liver Failure and Artificial Liver Support

CURRENT DESCRIPTORS: AO1 . Acute Liver Failure and Artificial Liver Support

TITLE: Safety and Tolerability of Ornithine Phenylacetate to Lower Ammonia in Acute Liver Failure: Preliminary report of the STOP-ALF Trial.

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ABSTRACT BODY:

Abstract Body: Background: No satisfactory method is currently available to lower ammonia (NH₃) to prevent or treat hepatic encephalopathy (HE) and cerebral edema in hospitalized patients with acute liver injury (ALI) or acute liver failure (ALF). Ornithine phenylacetate (OPA, OCR-002) effectively lowers NH₃ in animal models of ALF by trapping it as phenylacetylglutamine (PAGN) that is excreted in urine. STOP-ALF is a phase 2a trial to develop data on safety and tolerability of a 5-day infusion of OCR-002 in the setting of ALF/ALI and elevated NH₃ levels.

Methods: Patients with ALF or ALI and elevated NH₃ levels, >60 µmol/L, were enrolled at 8 US sites participating in the Acute Liver Failure Study Group (ALFSG) in two cohorts: normal/minimal renal dysfunction (Cr < 1.5, Cohort 1) and acute kidney injury (AKI, Cr ≥ 1.5 mg/dL, Cohort 2). Dosing of OCR-002 was open label, ascending dose, starting at 3.3 g/24hr by IV infusion, to a maximum dose of 10 gm/24 hr after 24 hr of infusion; for the 1st 3 pts, final dose = 3.3 gm/24hX5 days, then 6.7 gm/24hr for 3 and then 10 gm/24h for the remaining patients in each cohort. Plasma and urine PK and plasma NH₃ levels were recorded, in addition to safety labs, EKGs and frequent neurological checks.

Results: 30 patients have been enrolled, with current analysis based on 22 with PK data available; median age 32 yrs, 14/22 female, 16 were acetaminophen-related, 17 were considered evaluable, having received >72 hrs of OCR-002. The drug was well tolerated with only minor AE's attributed (nausea, headache). PK results showed mean phenylacetate (PAA) plasma levels of 29.0 and 62.5 µg/mL in Cohort 1 at the 2 higher dose levels, and 32.5 µg/mL for Cohort 2, dose level 2. These levels of PAA are below the optimal concentration for NH₃ removal. Overall survival was 73%, with deaths limited to advanced AKI patients and those unable to receive liver transplantation; 1 pt. required transplantation. NH₃ levels improved in most patients: median baseline NH₃=87 µmol/L to 54 µmol/L day 4, 0 hour. This ongoing trial is currently completing the final dose level; no toxic levels of PAA or PAGN have been reached.

Conclusion: OCR-002 was well tolerated without any safety signals and shows promise as adjunctive therapy in ALF. Higher doses of OCR-002 may be needed for optimal ammonia reduction in ALF.

Future studies to assess safety and efficacy of higher doses of OCR-002 will be pursued.

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Co-Author Disclosure Status

The following authors have completed their AASLD 2015 disclosure:: William Lee: Disclosure completed | R. Todd Stravitz : Disclosure completed | Robert Fontana: Disclosure completed | A. James Hanje: Disclosure completed | Holly Tillman: Disclosure completed | Kristen Clasen: No Answer. | Valerie Durkalski: Disclosure completed | Averell Sherker: Disclosure completed | Edward Doo: Disclosure completed | Brian Davis: Disclosure completed