



L-Ornithine phenylacetate (OP): A novel treatment for hyperammonemia and hepatic encephalopathy

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Summary Hepatic encephalopathy (HE) is a common neuropsychiatric complication of liver disease affecting about 20–30% patients with cirrhosis. HE may only affect quality of life (e.g. impairments in attention; coordination; driving ability), but in some patients this progresses to coma and death; defining mortality in those with acute liver failure. HE is thought to occur through accumulation of ammonia as a by-product of protein metabolism. In liver failure ammonia accumulates to toxic levels, resulting in ammonia-associated brain swelling. Presently, there is no proven therapy for HE though recent studies suggest that during liver failure, ammonia removal by skeletal muscle (by conversion to glutamine) can be manipulated; also that ammonia and amino acid metabolism should be viewed in terms of their interorgan relationship. This led us to develop a novel concept for ammonia removal. Preliminary studies provide the proof of concept that the combination of L-ornithine (amino acid) with phenylacetate, as L-ornithine phenylacetate (OP), reduces toxic levels of ammonia by (1) L-ornithine acting as a substrate for glutamine synthesis from ammonia in skeletal muscle and (2) phenylacetate excreting the ornithine-related glutamine as phenylacetylglutamine in the kidneys. As both L-ornithine and phenylacetate are already available for human use, data showing its usefulness in ammonia lowering could translate quickly into providing the much needed therapy for HE patients.

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Background

The term hepatic encephalopathy (HE) encompasses a spectrum of mental disturbances observed

in patients with liver disease. This may range from a 'minimal' reduction in quality of life to coma and death [1]. With advancing disease, cerebral edema develops secondary to astrocyte swelling [2,3]. Since Nencki and Pavlov described meat intoxication in portacaval shunted dogs over 100 years ago [4], ammonia has been considered central to the pathogenesis of HE [5]. The clinical importance of the role of hyperammonemia in patients with liver failure is no more evident than the observation that ammonia levels of >150 μmol/L predicts brain herniation, coma and death in patients with acute liver failure (ALF) [6].

Abbreviations: HE, hepatic encephalopathy; ALF, acute liver failure; CLD, chronic liver disease; GS, glutamine synthetase; LOLA, L-ornithine, L-aspartate; OP, ornithine, phenylacetate.

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The plasma ammonia concentration, its brain delivery and uptake of ammonia are increased in liver failure [7,8]. A proposed mechanism for the late stages of HE (intracranial hypertension and brain edema) involves the differential uptake of ammonia by astrocytes in the brain [9,10]. The enzyme glutamine synthetase (GS) (Fig. 1a) is found in high concentrations within astrocyte end-feet. These astroglial processes surround the brain microvessels of the blood brain barrier and swell in the presence of advanced hepatic encephalopathy. Despite significant astroglial changes, the barrier function remains intact; suggesting cytotoxic rather than vasogenic mechanisms predominate in the pathogenesis of hepatic encephalopathy [11]. Data from cell culture studies suggest that astrocyte swelling is related to astroglial ammonia uptake, driving GS to convert glutamate into the intracellular osmolyte glutamine, with accumulation of intracellular water [12]. Further studies suggest that the relative protection from intracranial hypertension and brain edema observed in cirrhosis (chronic liver disease, CLD) is related to a compensatory expulsion of weaker intracellular osmolytes (e.g. myoinositol, choline), because of a more gradual increase in plasma ammonia concentration [13]. This is in contrast to the frequent progression of hepatic encephalopathy in ALF which defines mortality; where it may be that dramatic shifts in plasma ammonia concentration outstrip such compensatory mechanisms.

At present there is no specific treatment of proven value for HE. A recent Cochrane meta-analysis finds the data for conventional treatment approaches for HE such as lactulose and orally administered non-absorbable antibiotics unsatisfactory [14,15]. Protein restriction, likewise may be more

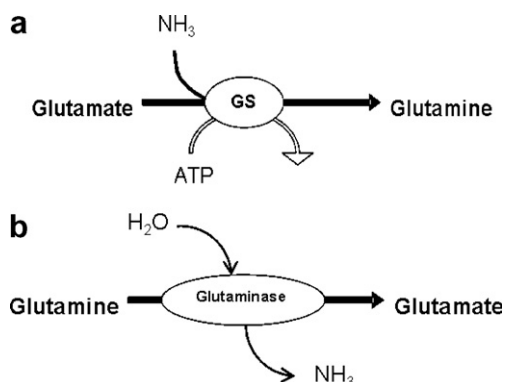


Figure 1 Schematics illustrating that glutamine is an intermediate ammonia (NH₃) sink. (a) Interaction of glutamate and ammonia by glutamine synthetase (GS) produces glutamine, whereas (b) the interaction of glutamine with glutaminase produces glutamate and ammonia.

harmful for cirrhotic patients and cannot be recommended [16]. This article focuses on recent studies that indicate that the metabolism of ammonia is complex and involves multiple organs, providing the basis of a novel approach to ammonia lowering in patients with cirrhosis.

Observations leading up to the hypothesis

Ammonia and amino acid metabolism in liver failure

The interrelationship between ammonia and amino acid metabolism in HE is not restricted to the brain. There is evidence of a more widespread disturbance in the metabolism of ammonia and glutamine in liver failure [17,18]. Glutamine can be both a sink for excess ammonia by combining with glutamate through GS activity (Fig. 1a), or as a source for ammonia release through glutaminase activity (Fig. 1b) [19]. Clearly the dominant locations of these enzymes and their down- or up-regulation can have a significant influence on circulating ammonia levels. Recent evidence highlight the importance of inter-organ ammonia and amino acid metabolism in this process (Fig. 2) [20,21].

Interorgan ammonia and amino acid metabolism in liver failure

Gut: Traditional hypotheses support the notion that ammonia is produced in the gut from bacterial breakdown of ingested proteins. However, recent studies suggest that a large proportion of the gut derived ammonia is produced metabolically [19–21], supported by the observation of substantial gut-derived ammonia production in germ free rats

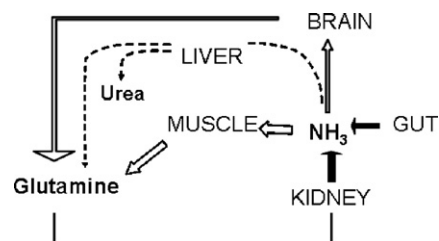


Figure 2 Schematic demonstrating the principal of interorgan ammonia and glutamine cycling, indicating the crucial role that the muscles play in ammonia metabolism in patients with liver failure. (This figure was published in Wright G, Jalan R. Management of hepatic encephalopathy in patients with cirrhosis. *Best Pract Res Clin Gastroenterol* 2007;21(1):95–110. Copyright Elsevier (2006).) (Nb: This figure is not meant to be quantitative).

[20]. Circulating glutamine (rather than luminal) is taken up by the enterocytes (especially in the jejunum and ileum), to form glutamate and ammonia, by the action of phosphate activated glutaminase [21,22]. Gut-derived ammonia passes through the liver via the portal vein.

Liver: Normally, ammonia is detoxified into urea by the fully functioning liver. In a diseased liver the impaired turnover of the urea cycle leads to a rise in circulating ammonia levels. The liver itself is a prominent site for glutaminase activity, particularly in the peri-portal hepatocytes. Hepatocyte glutaminase is also activated by ammonia and this may be related to the location of urea cycle enzymes in these cells. The peri-venous hepatocytes have GS. Ammonia efflux studies from the hepato-splanchnic region of post-absorptive healthy volunteers suggest that these enzymes can act in concert to easily accommodate rapid changes in systemic ammonia levels; when the hepatic-venous ammonia levels were between 20–40 mM, the ammonia flux was zero [23]. In contrast ALF patients have been shown to have increased ammonia efflux on a background of significantly elevated hepatic-venous ammonia levels.

Kidney: Renal metabolism also plays a role in ammonia homeostasis in which the kidney can act both to produce and remove ammonia as this organ has both GS and glutaminase [24]. The relative importance of these enzymes depends on blood ammonia levels; in liver failure the kidneys become a net ammonia excretor [21]. In addition, the contribution of the kidney is significantly effected by the presence of other factors impacting on function (e.g. diuretic therapy [25] and hepatorenal syndrome).

Muscle: The skeletal muscle GS is normally low but in liver failure gene expression, protein expression and the activity of GS was found to be up-regulated [22]. This observation suggests that the skeletal muscle could be an alternative therapeutic target for ammonia detoxification as muscle mass is relatively large. Therefore, supplying the muscle with glutamate can theoretically increase muscle glutamine efflux. As glutamate is not transported into muscle cells readily, glutamate can be provided to the muscle as L-ornithine.

Effects of L-ornithine L-aspartate for the treatment of hyperammonemia and HE

The concept of delivery of L-ornithine to the muscle is the basis of the agent L-ornithine L-aspartate (LOLA) for ammonia lowering in cirrhosis. LOLA provides L-ornithine and L-aspartate as substrates

for glutamate production. Randomized controlled trials (RCT's) of LOLA administration to patients with chronic liver failure, hyperammonemia and subclinical HE, have reported reductions in plasma ammonia and improved psychometric test scores [26,27]. Studies on animal models of liver failure with brain swelling, would suggest that these benefits also extend to advanced HE [28]. However, the results of the use of LOLA are not universally positive, with a number of apparently negative trials yet to be published.

Some of our recent observations challenge the view that LOLA produces sustained reduction in ammonia levels. We studied 8 patients with cirrhosis following treatment with an infusion of 40 g/day LOLA (Fig. 3). We observed an initial mild reduction in ammonia concentration with a concomitant rise in glutamine. This increase in glutamine due to the effects of the infused LOLA spontaneously reduced over the following days with recurrence of hyperammonemia once LOLA was discontinued. This rebound increase in ammonia was associated with recurrence of severe HE in 4 of the 8 patients. Therefore, we can hypothesize that any initial reduction of plasma ammonia with LOLA may be only transient, leading to a significant rise in glutamine levels which eventually becomes a source for ammonia-generation by the kidney and gut through the effects of glutaminase [20]. In addition, the role of aspartate remains unclear. Aspartate infusions in animals were not shown to result in a reduction in ammonia levels [29] and infusion of LOLA, resulted in an accumulation of Aspartate, increasing from 72 $\mu\text{mol/L}$ to 354 $\mu\text{mol/L}$ indicating that aspartate was unlikely to be biologically active precursor of glutamate/glutamine and that L-ornithine was likely to be the active component of LOLA.

Treatment of other hyperammonemic diseases due to defects of the urea cycle

Phenylbutyrate (*converted to phenylacetate in vivo*) has been used for the hyperammonemia which occurs with urea cycle enzyme deficiencies [30]. Phenylacetate covalently combines with the glutamine derived from glutamate to make phenylacetylglutamine which is excreted by the kidneys. In such metabolic disorders elevated glutamine levels act as the substrate for phenylacetate, thereby removing it as a substrate for ammonia-generation. However, in cirrhosis the plasma glutamine concentrations remain normal, and it is

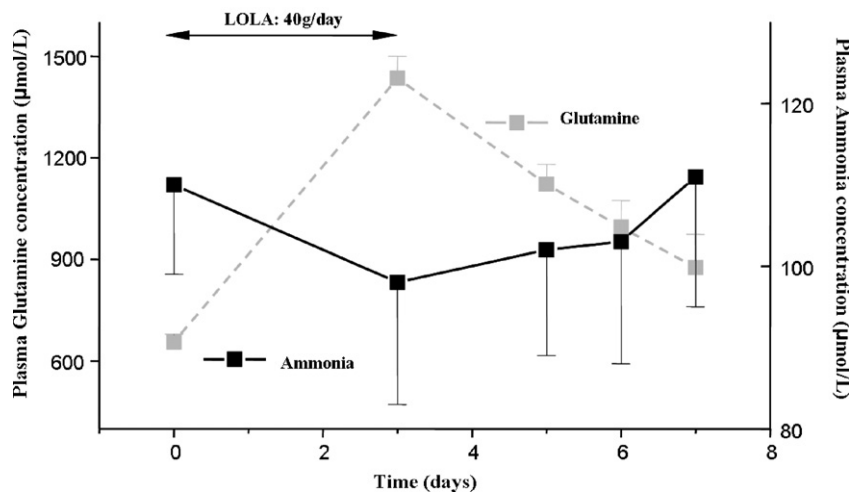


Figure 3 Represents differences in plasma ammonia and glutamine concentrations over time in 8 patients with cirrhosis who were treated with LOLA infusions over the first 3 days. The initial reduction in ammonia (represented as a solid black line) was associated with a concomitant rise in glutamine (represented as a dashed line), which regenerated ammonia once LOLA was stopped. This rebound increase in ammonia was associated with recurrence of severe hepatic encephalopathy in 4 of the 8 patients.

unlikely that in this situation phenylacetate alone will be useful.

The hypothesis

The above studies of inter-organ ammonia trafficking and the lessons from LOLA observations and the current use of phenylacetate (often administered as phenylbutyrate) to treat urea cycle enzyme disorders have led up to the hypothesis. Currently available studies support the view that L-ornithine can be taken up by the muscle to produce glutamine (in the process consuming 1 molecule of ammonia) and phenylacetate is effective in removing excess glutamine (in patients with urea cycle enzyme abnormalities).

Hypothesis: The concomitant administration of L-ornithine and phenylbutyrate or phenylacetate in liver failure produces a sustained reduction in ammonia concentration through (Fig. 4).

1. provision of glutamate (by transamination of ornithine) for detoxification of ammonia to glutamine;
2. excretion of the glutamine thus formed as phenylacetylglutamine (in combination with phenylacetate) in the urine.

This would have the advantage that the ammonia trapped as glutamine will not be available for later return to the circulation, resulting in net removal and reduction in ammonia concentration.

We undertook a proof of concept study to establish if OP can reduce ammonia in patients with cirrhosis and thereby improve the severity of encephalopathy. In an open labelled preliminary study, we included 8 patients with cirrhosis and hyperammonemia, matched for the severity of liver disease. They were treated with (i) Placebo; (ii) L-ornithine alone: 20 g in 500 ml, 5% Dextrose (IV, 4 h); (iii) phenylbutyrate: 10 g twice daily, orally; (iv) ornithine + phenylbutyrate: at above doses, for 3 days and the patients followed for 5 days. The drug was tolerated well in each of the groups and no adverse events were observed. The ammonia data are summarized in Fig. 5. Both patients in the OP group had improved their enceph-

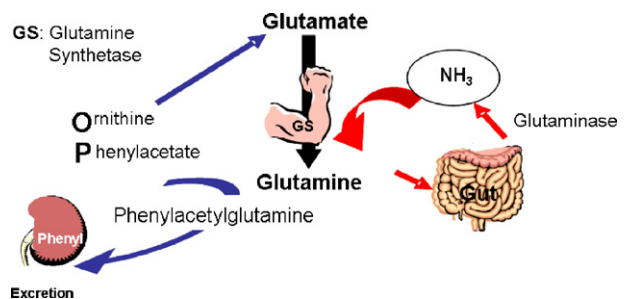


Figure 4 Schematic representation of the L-ornithine-phenylbutyrate (OP) hypothesis demonstrating that the hyperammonemia of liver failure rise is attenuated by conversion of the L-ornithine induced glutamine to phenylacetylglutamine in the kidney with its eventual excretion.

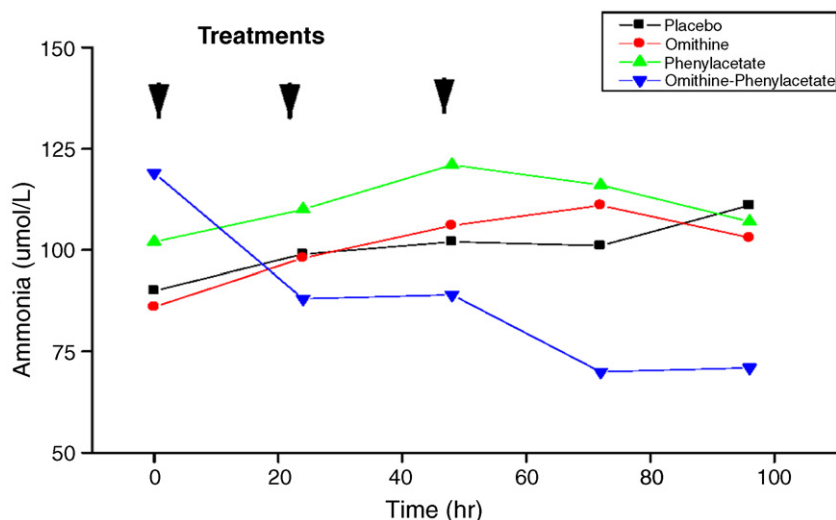


Figure 5 Mean venous ammonia concentrations in the 4 groups showing a reduction in the ammonia concentration in the OP treated patients by Ca. 30%. The mean ammonia levels remained largely unchanged over the period of treatment in the phenylacetate group. In the L-ornithine alone and placebo groups, the ammonia concentration increased from baseline values.

alopathy score by 2 grades by day 3, which was not observed in any of the other 6 patients.

Discussion

Our hypothesis provides the rationale for combining L-ornithine with phenylacetate to treat hyperammonemia in patients with cirrhosis. We also provide the proof of concept that OP produced a synergistic effect to simultaneously increase muscle detoxification, and reduce gut production of ammonia, resulting in attenuation in systemic hyperammonemia. In liver disease patients, the safety of L-ornithine has been previously demonstrated. Also, phenylacetate/phenylbutyrate is considered safe and beneficial as an intervention to reduce hyperammonemia in urea cycle disorders. Therefore, the potential to test OP in man may only be a short series of confirmatory experiments away.

Disclaimer

UCL holds the patents for the L-ornithine phenylacetate [PCT GB2005/004539; WO2006/056794].

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