

Targeting Ammonia & Inflammation With L-Ornithine, Phenylacetate (OP) And Infliximab For The Treatment Of Advanced Hepatic Encephalopathy

Gavin Wright, Vanessa Stadlbauer, Vairappan Balasubramaniyan, Nathan Davies and Rajiv Jalan*

Institute of Hepatology, University College London, London, United Kingdom. WC1E 6HX

Background

With acute liver failure (ALF) worsening hyperammonemia may result in progressive hepatic encephalopathy (HE), culminating in cytotoxic brain oedema, coma or death. Infection or aseptic inflammation may synergistically modulate the cerebral effects of ammonia. In cirrhosis, inflammation may rapidly worsen HE and induce acute-on-chronic liver failure (AoCLF), with features similar ALF. Interventions targeting either hyperammonemia or inflammation have been shown to independently limiting progression of HE, but the therapeutic effect of combining ammonia-lowering and anti-inflammatory agents has not been studied.

L-Ornithine Phenylacetate (OP) is a novel synergistic combination that has been found to produce sustained reduction in ammonia (in both large and small animal models) by L-ornithine acting as a substrate for glutamine synthesis (in skeletal muscle) thereby detoxifying ammonia, and the phenylacetate excreting the ornithine-derived glutamine as phenylacetylglutamine in the urine (Figure 1).

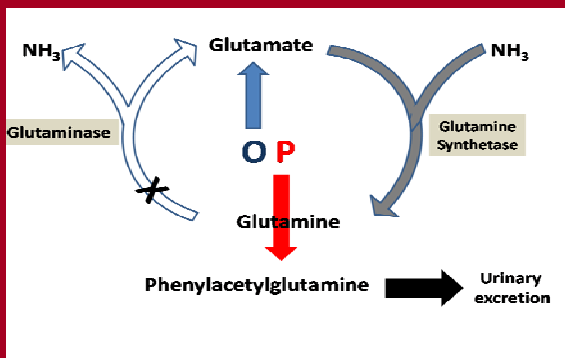


Fig. 1. Schematic demonstrating the glutamate–glutamine cycling and the hypothesized mechanism of action of the synergy between L-ornithine and phenylacetate (OP) in reducing ammonia concentration in liver failure.

L-ornithine acts as a substrate for glutamine synthetase, providing 2 moles of glutamate for every 1 mole of ornithine. For every mole of glutamate produced, 1 mole of ammonia is detoxified to 1 mole of glutamine in skeletal muscle. Glutamine can then combine with phenylacetate to generate phenylacetylglutamine which can then be excreted out through the kidneys.

Aims

To establish if treating both hyperammonemia (with the ammonia-lowering therapy OP) and inflammation (using Infliximab, a chimeric monoclonal antibody against the proinflammatory cytokine, TNF α) provides therapeutic synergism; by preventing bacterial lipopolysaccharide (LPS) induced cerebral oedema in bile-duct ligated (BDL) cirrhotic rats.

Methods

Sprague-Dawley rats were studied 4 weeks post BDL or sham-operation (sham). BDL rats were randomised to 3 days of intraperitoneal (IP) injections of OP (0.6g/kg) and/or 24 hours before termination 10mg/kg of IP Infliximab, or saline. Three hours before termination, all BDL rats received IP LPS (1mg/kg). Arterial ammonia, plasma biochemistry, regulatory plasma cytokines, consciousness and brain water were analysed (1) Sham 2) BDL + saline 3) BDL + LPS 4) BDL + LPS + OP, 5) BDL + LPS + Infliximab and 6) BDL + LPS + OP + Infliximab.

Results

Compared to sham, BDL was associated with significantly higher arterial ammonia ($p < 0.05$), TNF α and IL-6 plasma levels ($p < 0.01$, respectively) and a trend to increased brain water. Addition of LPS significantly worsened coma stage, brain water ($p < 0.05$) and augmented TNF α and IL-6 levels. Giving OP to LPS-treated BDL rats significantly reduced arterial ammonia and brain water ($p < 0.05$; respectively). Infliximab significantly reduced TNF α and IL-6 ($P < 0.01$) without a significant reduction in brain water. Despite improved coma score and brain water with co-administered OP and Infliximab compared to either therapy alone, this was not significantly different to just OP.

Conclusions

Suppression of TNF α dependent pathways by Infliximab failed to provide therapeutic synergy in addition to the significant ammonia-lowering effect of OP in reducing the catastrophic consequences of LPS in BDL rats. However, though hyperammonemia remains the central target in HE, as addition of Infliximab proved superior to lone therapy (albeit non-statistical), better targeting of inflammatory processes may yet prove synergistic.

Data

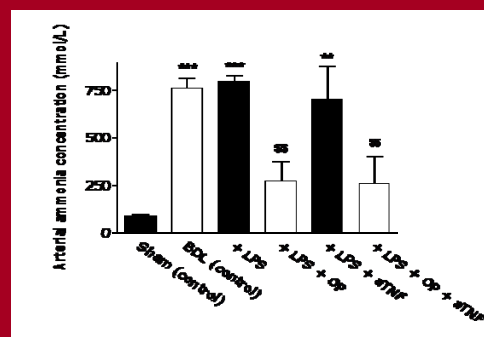
	Sham	BDL	BDL + LPS	BDL + LPS + OP	BDL + LPS + aTNF	BDL + LPS + OP + aTNF
Frontal brain water (%)	78.0 \pm 0.1	78.7 \pm 0.2	81.3 \pm 0.6*	79.2 \pm 0.4 ^s	80.1 \pm 0.4	78.5 \pm 0.3 ^s
Arterial ammonia (μ mol/l)	86 \pm 12	760 \pm 55***	796 \pm 32***	269 \pm 108 ^s	706 \pm 169**	258 \pm 143 ^s
Bilirubin (mol/l)	16 \pm 2.6	206 \pm 3	212 \pm 6	200 \pm 9	195 \pm 9	180 \pm 18
Creatinine (mol/l)	21 \pm 6.5	22 \pm 1	24 \pm 3	30 \pm 2	26 \pm 2	28 \pm 0
Urea (mmol/l)	5.5 \pm 0.4	5 \pm 0.2	5 \pm 0.6	6 \pm 0.6	6 \pm 0.3	5 \pm 0.6
Albumin (g/l)	39 \pm 2	35 \pm 2	32 \pm 1	35 \pm 1	32 \pm 1	26 \pm 1
Total protein (g/l)	53 \pm 2.8	46 \pm 3	48 \pm 2	45 \pm 2	45 \pm 2	54 \pm 3
Sodium (mmol/l)	137 \pm 4	139 \pm 2	138 \pm 3	140 \pm 5	138 \pm 1	141 \pm 3
Potassium (mmol/l)	5.4 \pm 1.0	4.9 \pm 0.2	4.8 \pm 0.6	5.1 \pm 0.2	4.2 \pm 0.2	5.9 \pm 0.5
Chloride (mmol/l)	105 \pm 4	104 \pm 1	97 \pm 2	108 \pm 3	105 \pm 2	106 \pm 2
Osmolarity (mOsm/l)	285 \pm 19	281 \pm 20	279 \pm 18	284 \pm 20	280 \pm 12	285 \pm 28

Data are expressed as mean \pm standard error of mean (SEM) Symbols represent: - * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to Sham-operated control rats; ^s $p < 0.05$, ^{ss} $p < 0.01$ and ^{sss} $p < 0.001$ compared to respective saline treated control rat.

Abbreviations: Sham, Sham-operated; BDL, bile duct ligation; LPS, lipopolysaccharide and aTNF, Infliximab.

	Sham (control)	BDL	BDL + LPS	BDL + LPS + OP	BDL + LPS + aTNF	BDL + LPS + OP + aTNF
Plasma TNF- α (pmol/L)	0.0 \pm 0.0	70 \pm 6	1505 \pm 500**	2885 \pm 617***	1828 \pm 294**	98 \pm 15
Plasma IL-6 (pmol/L)	0.0 \pm 0.0	0.0 \pm 0.0	1517 \pm 58**	3845 \pm 617***	3701 \pm 389***	788.8 \pm 190 ^{ss}
Plasma IFN- γ (pmol/L)	0.0 \pm 0.0	10 \pm 3	24 \pm 6	19 \pm 9	17 \pm 9	180 \pm 18

Arterial ammonia: Shows that compared with Sham-operated controls, there was a significant rise in arterial ammonia in BDL rats (*** $p < 0.01$), with no additional effect with LPS. Following administration of OP (\pm Infliximab), there was a significant reduction in arterial ammonia concentration from baseline at 3 hours (^{ss} $p < 0.01$, respectively) and no different to Sham controls.



Frontal cortex brain water: Frontal cortex brain water content: Shows that compared with Sham-operated controls, there was a non-significant rise in frontal cortex water content in BDL rats, which was significantly augmented by LPS (* $p < 0.05$). Administration of OP resulted (\pm Infliximab) in a significant reduction in brain water compared to LPS treated BDL rats (^s $p < 0.05$). Infliximab had a non-significant effect on brain water.

