

Treatment of hyperammonaemia with OCR-002 (L-ornithine phenylacetate) reduces portal pressure by modulating hepatic NFkB and hepatic eNOS activity in cirrhotic rats

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Background

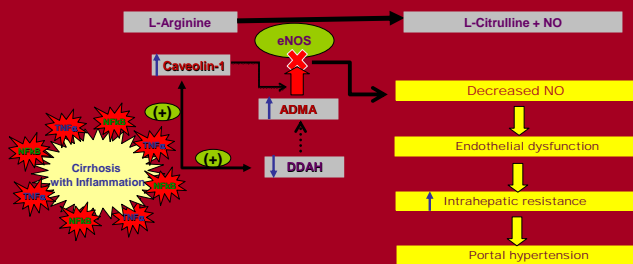
✦ In astrocyte cultures, hyperammonaemia induces oxidative stress and NFkB and promotes inflammation (Norenberg 2008)

✦ Hyperammonaemic animals manifest increased TNF α levels (Jalan 2007), and reduced bioavailability of brain nitric oxide (Felipo 2007), which can be corrected by reducing inflammation with ibuprofen.

✦ In cirrhosis, severity of portal hypertension is associated with increased hepatic inflammation, reduced eNOS activity and increased endogenous NOS inhibitors, Caveolin-1 and asymmetric-dimethylarginine (ADMA) (Mookerjee 2007)

✦ OCR-002 is a novel salt made up of equimolar concentration of L-ornithine and Phenylacetate, shown to be effective in:

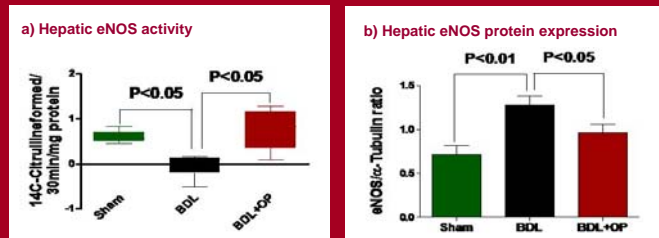
- reducing ammonia and brain water in cirrhotic rats (Davies et al. 2009)
- attenuating rise in ammonia and intracranial pressure in devascularised acute liver failure pigs (Ytrebo et al, 2009)



Results

| Parameters | Sham | BDL | BDL+OP |
|----------------------------------|------------------|---------------------|--------------------------------|
| Ammonia ($\mu\text{mol/L}$) | 55.86 \pm 4.05 | 222.3 \pm 24.01** | 127.1 \pm 47.8† |
| ALT (U/L) | 26.88 \pm 2.61 | 159.8 \pm 10.81** | 108.3 \pm 5.66† |
| Bilirubin ($\mu\text{mol/L}$) | 32.52 \pm 2.43 | 304.9 \pm 25.97** | 267.7 \pm 14.5 ^{NS} |
| Creatinine ($\mu\text{mol/L}$) | 23.07 \pm 0.94 | 40.88 \pm 2.37* | 32.38 \pm 1.44 \S |
| TNF- α (pg/ml) | 121.9 \pm 46.4 | 822.9 \pm 203.1* | 270.5 \pm 59.4 \S |

Table - Effect of OCR-002 (OP) on arterial ammonia and plasma biochemistry of BDL rats. Statistical significance was calculated using student t' test Mann Whitney comparisons test. * - P<0.01 versus sham; ** - P<0.001 versus sham; † - P<0.01 versus BDL; \S - P<0.05 versus BDL; NS-no significance.



eNOS activity is significantly decreased in BDL animals compared to sham (p<0.05) despite increased eNOS protein expression (p<0.01). Following treatment with OCR-002, eNOS activity reverts to sham levels, with similar normalisation of eNOS protein expression (p<0.05)

Aims

✦ This study was designed to test the hypothesis that reducing ammonia concentrations with OCR-002 (L-ornithine phenylacetate) will:

- reduce systemic and hepatic inflammation
- increase intrahepatic NO availability through effects on the NO regulatory pathways of caveolin-1 and DDAH-ADMA
- thereby reduce portal pressure

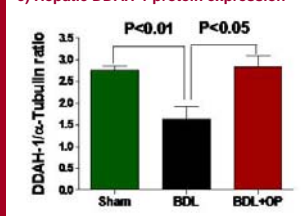
Methods

Animals: Four weeks after BDL and sham surgery in Sprague-Dawley rats, BDL rats were given i.p. OCR-002 3g/kg twice a day or vehicle alone (n=14/group), and treated for 5 days .

Measurements: After the 5th treatment day, all rats underwent direct portal pressure measurement under anaesthesia prior to sacrifice. Plasma and liver tissue was harvested for subsequent analysis:

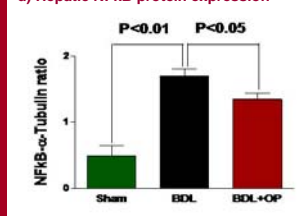
- Plasma ammonia and biochemistry were measured using a Cobas-Integra analyser
- Plasma TNF α was measured by FACS cytometric bead array
- eNOS activity was determined radiometrically
- Protein expression for eNOS, DDAH-1, caveolin-1, and NFkB were measured using standard Western Blotting techniques.

c) Hepatic DDAH-1 protein expression



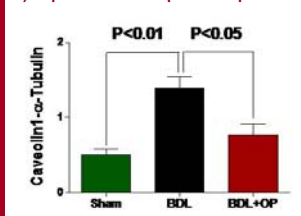
DDAH-1 protein expression is significantly higher in sham animals compared to BDL (p<0.01). Following OCR-002 administration to BDL rats, DDAH-1 expression increases significantly and reverts to sham levels (p<0.05)

d) Hepatic NFkB protein expression



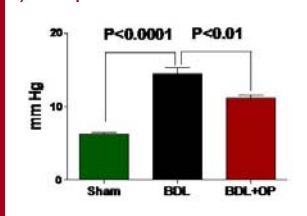
Protein expression of NFkB is significantly elevated in BDL rats compared to sham (p<0.01). OCR-002 treatment produces a marked reduction in NFkB expression compared to BDL (p=0.05).

e) Hepatic caveolin-1 protein expression



The expression of caveolin-1 is significantly elevated in BDL rats compared to sham (p<0.001). Following intervention with OCR-002 Caveolin-1 expression decreases significantly (p<0.05) compared to BDL alone.

f) Portal pressure



Portal pressure is significantly increased in BDL rats compared to sham (p<0.0001). OCR-002 treatment results in a 30% reduction of portal pressure as compared to BDL (P<0.01)

Conclusions

➢ This study demonstrates for the first time that treatment of hyperammonaemia with OCR-002 in a clinically relevant model of cirrhosis reduces portal pressure significantly by:

- Reducing systemic, hepatic and NFkB inflammatory signalling
- Restoring hepatic eNOS activity by favourably modulating the inhibitory pathways of DDAH-ADMA and caveolin-1
- Our data provides the rationale for evaluating OCR-002 in the treatment of portal hypertension.

