

# REDUCTION IN HYPERAMMONAEMIA WITH OCR-002 (L-ORNITHINE PHENYLACETATE) IN BILE-DUCT-LIGATED (BDL) CIRRHOTIC RATS RESTORES BRAIN eNOS ACTIVITY BY MODULATING THE DDAH-ADMA PATHWAY

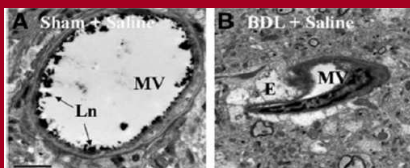
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## Background

- Hepatic encephalopathy (HE) - serious complication of cirrhosis
- Ammonia is central in the pathogenesis of HE
  - Mechanism by which ammonia produces neuropsychological dysfunction is not clear
  - In isolated astrocytes, ammonia produces oxidative stress and increased NFκB (Norenberg et al; J Neurochem, 2009 & J Neurosci Res, 2009)

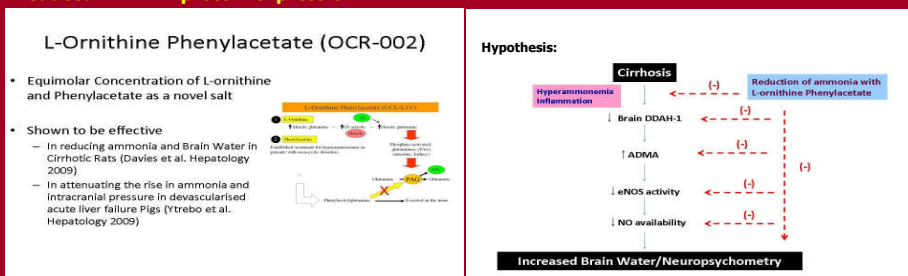
- In cirrhosis, cerebral blood flow is reduced (Iversen et al., Gastroenterology; 2009)

➤ The electron micrograph shows a very constricted brain micro-vessel (MV) in a cirrhotic rat compared with a Sham operated control (Wright et al., Hepatology 2007)



## Regulation of brain NO through the DDAH-ADMA pathway

- Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of eNOS
- ADMA levels are tightly regulated by the enzyme dimethylarginine-dimethylaminohydrolase-1 (DDAH-1)
- Inflammation is known to inhibit DDAH-1 enzyme activity
- In liver failure, we have shown (Balasubramaniyan et al., AASLD 20008 & BASL 2009)
  - Reduced Hepatic eNOS activity and NO availability
  - Increased Hepatic ADMA
  - Reduced DDAH-1 protein expression



## Aims

The aim of this study was to determine whether reduction in ammonia concentration with OCR-002 impacts upon the NO pathway. The questions this study is designed to address are

- is eNOS activity reduced in cirrhotic brains?
- are ADMA levels increased and dimethylarginine-dimethylaminohydrolase (DDAH1, metabolizes ADMA) decreased in cirrhotic brains?
- Does the administration of OCR-002 to BDL cirrhotic animals result in alteration of:
  - Arterial and Brain Ammonia concentrations and Brain water content
  - Arterial and Brain Cytokine levels
  - eNOS protein and activity
  - ADMA levels and DDAH-1 protein expression

## Methods

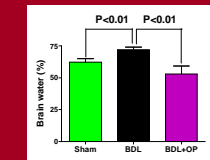
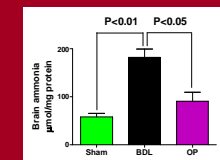
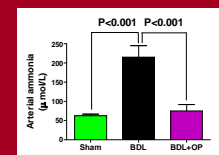
**Animals:** Sham operated Sprague-Dawley rats (n=10) and BDL rats (n=10) were compared four weeks after BDL surgery, and in an additional BDL group (n=6), after administration of 3g/kg i.p. OCR-002 (OP) twice a day for 5 days.

### Measurements:

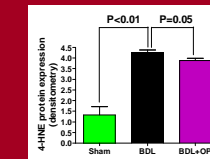
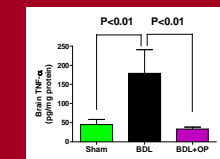
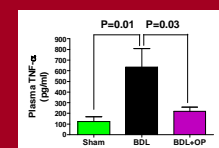
Plasma ammonia was measured using a Cobas-Integra, brain water was measured using the dry weight technique, TNFα was measured by FACS cytometric bead array, plasma and brain ADMA were measured using LC-MS/MS, eNOS and DDAH activity were determined radiometrically, protein expression for eNOS, DDAH-1, and 4HNE were measured by western blotting.

## Results

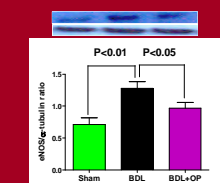
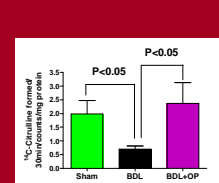
Treatment of BDL rats with OCR-002 reduces arterial and brain ammonia and normalisation of brain water



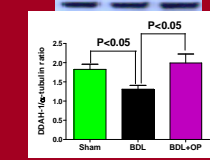
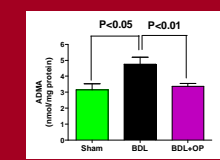
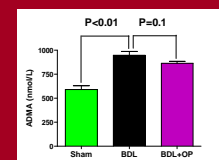
Treatment of BDL rats with OCR-002 reduces arterial and brain TNF-α and oxidative stress marker



Treatment of BDL rats with OCR-002 restore eNOS activity and protein expression



Treatment of BDL rats with OCR-002 restore plasma and brain ADMA and DDAH-1 protein expression



## Conclusions

- Treatment of Hyperammonemia in Cirrhotic rats with OP reduces:
  - Arterial and cerebral ammonia and brain water content
  - Plasma and brain TNF-α and oxidative stress marker
- These are associated with
  - Restoration of eNOS protein expression and increased eNOS activity
  - These effects on eNOS are mediated by restoration of the DDAH/ADMA axis
- Our studies support the hypothesis that either ammonia and/or its effects on cell swelling are critically related to inflammation and NO signalling in the brain which may have important therapeutic consequences

Acknowledgement: OCR-002 supplied by Ocera Therapeutics, San Diego, CA

