

## 827 PROTECTIVE EFFECTS OF ERYTHROPOIETIN IN RATS WITH CIRRHOTIC CARDIOMYOPATHY

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**Background:** Erythropoietin (EPO) is a major regulator of erythropoiesis. In addition, EPO receptor 1 (EPOR1) is found in rat heart. EPO can protect cardiomyocytes from ischemic injury, attenuate myocardial inflammation by suppression of tumor necrosis factor (TNF $\alpha$ ) and improve cardiac contractile function. Similar to ischemic cardiomyopathy, cirrhotic cardiomyopathy shows decreased beta-adrenoceptors ( $\beta$ -AR) and decreased systolic and diastolic responses to stress stimuli. To date, no medical therapies have been shown to improve contractile function in cirrhotic cardiomyopathy. Therefore, it is crucial to explore possible new therapeutic agents for this condition. **Methods:** Rats were divided into 4 groups, sham control; sham + EPO; bile duct ligation (BDL); and BDL + EPO (n=6 in each group), and were studied 4 wk after surgery. EPO (1000 U/kg body weight every other day, i.p.) or equivolumic normal saline control injections were administered starting 10 days before the study date. Samples were collected for the measurement of TNF $\alpha$ , and EPOR1 expression. In separate groups of rats, isolated cardiomyocytes were subjected to contractile and relaxation function studies. **Results:** TNF $\alpha$  was significantly increased in BDL heart compared with sham control (345  $\pm$  48 vs 250  $\pm$  18 pg/mg protein) and sham+EPO group (p<0.01). EPO significantly decreased TNF $\alpha$  expression in the BDL group (345  $\pm$  48 vs 257  $\pm$  32 pg/mg protein, p<0.01). There was no statistical difference between the sham control, sham + EPO and BDL + EPO groups. Western blot analysis showed that EPOR1 protein expression was significantly increased in the left ventricles from BDL compared with those from sham controls (P < 0.01). Isoproterenol-stimulated maximal systolic velocity in isolated cardiomyocytes was significantly decreased in cirrhotic rats compared with sham control (4.26  $\pm$  1.09 vs 7.82  $\pm$  2.27  $\mu$ m/s, p<0.05). EPO significantly reversed the depressed contractility in the cirrhotic cardiomyocytes (4.26  $\pm$  1.09 vs 8.26  $\pm$  1.30  $\mu$ m/s, p<0.01). Maximal diastolic velocity was also slower in cirrhotic rats compared with the sham group (2.21  $\pm$  0.88  $\mu$ m/s vs 5.53  $\pm$  1.57, p<0.05). EPO corrected the diastolic velocity in BDL hearts (2.21  $\pm$  0.88 vs 6.06  $\pm$  0.88  $\mu$ m/s, p<0.01). **Conclusions:** EPO receptors were increased in cirrhotic hearts. EPO treatment significantly decreased TNF $\alpha$  concentration and reversed the impaired systolic and diastolic function in cirrhotic cardiomyocytes. These results suggest that erythropoietin mediates at least part of its cardiostimulant effect by inhibiting the TNF $\alpha$  pathway, and may be a potential treatment for cirrhotic cardiomyopathy.

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## 828 EFFECT OF LONG-TERM INHIBITION OF ACID GASTRIC SECRETION ON GASTRIC PH AND ON BACTERIAL TRANSLOCATION IN CIRRHOTIC RATS

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**Background:** Bacterial translocation, as a consequence of intestinal bacterial overgrowth, plays an important role in the pathogenesis of bacterial infections in cirrhosis. Long-term inhibition

of acid gastric secretion promotes intestinal bacterial overgrowth and could favour bacterial translocation. **Aim:** to evaluate the effect of long-term inhibition of acid gastric secretion on gastric pH and on bacterial translocation in cirrhotic rats with or without ascites. **Material and Methods:** Cirrhotic rats with and without ascites induced by oral carbon tetrachloride were randomized to be treated with a daily subcutaneous injection of placebo, ranitidine (100mg/kg) or pantoprazole (8mg/kg) during two weeks. A control non-cirrhotic group were also randomized. The first day of the study (basal) and the previous day to the laparotomy a continuous 2 hour pH-metry under anesthesia was performed to evaluate the effect of antisecretory therapy. A laparotomy was performed to obtain samples of blood, mesenteric lymph nodes, ascites, pleural fluid, spleen, liver and cecal stools for culture. **Results:** Mortality was significantly higher in cirrhotic rats with ascites than in nonascitic cirrhotic rats (p<0.01) and in control rats (p<0.001) during the study, but no differences were observed in rats treated with placebo vs antisecretory drugs. Ranitidine and pantoprazole significantly increased gastric pH compared to rats treated with placebo (p<0.001). Administration of antisecretory drugs did not modify the incidence of bacterial translocation in nonascitic rats and control rats. In contrast, a higher incidence of bacterial translocation was observed in ascitic rats treated with ranitidine (7/11, p<0.05), pantoprazole (7/12, p=0.1) or any antisecretory therapy (14/23, p<0.05) compared to placebo treated ascitic rats (3/14) and to nonascitic rats treated with the same antisecretory drug (p<0.05, p=0.06 and p<0.01, respectively). Despite gastric pH previous to laparotomy was significantly higher in cirrhotic rats treated with pantoprazole than with ranitidine (p<0.001), the incidence of bacterial translocation was similar in both groups. **Conclusions:** Inhibition of acid gastric secretion increased acid gastric pH in control and cirrhotic rats with or without ascites, but the incidence of bacterial translocation only in cirrhotic rats with ascites. A similar incidence of bacterial translocation was observed in ascitic cirrhotic rats treated with any antisecretory therapy.

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## 829 L-ORNITHINE PHENYLACETATE REDUCES ARTERIAL AMMONIA, IMPROVES BRAIN OSMOLYTES AND REDUCES BRAIN WATER IN A BILE DUCT LIGATED RAT MODEL OF CIRRHOSIS

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**Introduction:** Treatment of hyperammonemia and HE in cirrhosis are unmet clinical needs. In cirrhosis, the muscles can detoxify ammonia into glutamine. This concept has been exploited by the drug L-ornithine L-aspartate, but the glutamine thus produced regenerates ammonia in the gut and kidneys by the action of glutaminase. This study tests the hypothesis that L-ornithine-phenylacetate (OP) would reduce hyperammonemia by L-ornithine acting as a substrate for glutamine (Gln) synthesis (from ammonia) in skeletal muscle, and phenylacetate com-

binning with this Gln to form phenylacetylglutamine (PAG) which is then excreted in urine. **Methods:** Rats were studied 6 weeks after bile-duct ligation (BDL) or Sham-operation. The BDL rats were treated for 1 week with high protein/ammoniogenic diet. Three hours before termination, intraperitoneal administrations of either, phenylbutyrate (pro-drug; 0.3gm/Kg) or L-ornithine (0.3gm/Kg) alone; 'OP' (0.3 gm/Kg of each) or placebo was given. Hourly arterial samples were collected for ammonia and urine for PAG. Samples collected at termination were measured for; Brain water (dry weight); Brain tissue osmolytes (1H-NMR spectroscopy); and muscle glutamine synthetase (GS) activity. **Results:** Compared to Sham-operated rats, BDL rats had significantly increased plasma ammonia ( $p < 0.001$ ) and glutamine/myo-inositol (Gln/ml) ratios ( $p < 0.001$ ); brain water content ( $p < 0.01$ ) and perivascular edema. Administration of OP to BDL rats significantly reduced plasma ammonia (2-way ANOVA,  $p < 0.05$ ) and brain water ( $p < 0.05$ ) versus the other 3 groups and ameliorated low-grade edema histologically. The Gln/ml ratio ( $p < 0.001$ ) was lowered to values not significantly different to Sham-operated rats which was not observed with L-ornithine or phenylbutyrate administration alone. These changes were associated with significantly increased urinary PAG and muscle GS activity ( $p < 0.01$  and  $p < 0.05$ , respectively). **Conclusion:** The results show that OP reduces ammonia significantly in cirrhosis by increasing muscle ammonia detoxification and elimination by the ornithine related glutamine as urinary PAG. Also, by reducing plasma ammonia, OP leads to normalisation of brain osmolytes and brain water content suggesting a reduction of edema. These observations provide the rationale for further development of OP as a drug for clinical trials in patients with HE.

|                         | Sham-operated (n=7) | BDL + placebo (n=7) | BDL + OP (n=8) |
|-------------------------|---------------------|---------------------|----------------|
| Brain water content (%) | 76.1 ± 0.4          | 77.90 ± 0.43##      | 76.01 ± 0.69#  |
| GLN/ml ratio            | 0.83 ± 0.06**       | 1.32 ± 0.02         | 0.95 ± 0.04**  |
| Urinary PAG (mm/L)      | 0.05 ± 0.16         | 0.5 ± 0.14          | 11.46 ± 3.26#  |

#  $p < 0.05$ , ##  $p < 0.01$  versus Sham-operated rats

\*  $p < 0.01$ , \*\*  $p < 0.001$  versus BDL rats

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### 830

#### EVALUATION OF PULMONARY HYDROGEN SULFIDE GENERATION IN EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)

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**Introduction:** Hydrogen sulfide (H<sub>2</sub>S) has emerged as a third endogenous gaseous signaling transmitter and a vasodilator, alongside nitric oxide (NO) and carbon monoxide (CO). Deficient hepatic H<sub>2</sub>S production, due to a reduction in cystathionine  $\gamma$ -lyase (CSE) the main enzyme forming H<sub>2</sub>S in peripheral tissues and vascular system, in experimental biliary cirrhosis (common bile duct ligation, CBDL) has recently been postulated to contribute to portal hypertension. CBDL animals also develop intrapulmonary vasodilatation and HPS and NO and CO contribute to this effect. However, the potential role of H<sub>2</sub>S in the

pulmonary vascular abnormalities of HPS has not been explored. **Aim:** To characterize pulmonary and hepatic CSE expression and H<sub>2</sub>S generation in experimental HPS in relation to non-cirrhotic portal hypertension (partial portal vein ligation, PVL). **Methods:** Control, 1, 2, 3 and 4 week CBDL and 3 week PVL Sprague-Dawley rats underwent evaluation. The physiologic features of HPS were evaluated using ABGs and microsphere shunting. Western blot analysis and quantitative RT-PCR were used to assess CSE protein and mRNA expression in lung and liver samples. H<sub>2</sub>S generation in tissue homogenates were measured using a respirometer chamber and recorded with a polarographic H<sub>2</sub>S sensor (PHSS) connected to a multichannel analyzer (Apollo 4000, WPI, Sarasota, FL). **Results:** CBDL animals, but not PVL animals developed physiologic alterations of HPS beginning within 2 wk after ligation. Pulmonary CSE expression decreased significantly within 2 wk relative to control and progressed over time (70% reduction in 4 wk CBDL vs control,  $p < 0.05$ ). These changes were accompanied by parallel reductions in lung H<sub>2</sub>S generation (control and 4 wk CBDL as  $4.6 \pm 0.9$  and  $1.6 \pm 0.2$  pmol/sec/mg protein,  $p < 0.05$ ). The decrease in CSE expression and H<sub>2</sub>S production also occurred over a similar time frame in CBDL liver. In contrast, PVL animals had no decrease in either CSE expression or H<sub>2</sub>S production in lung or liver relative to control, despite a similar degree of portal hypertension. **Conclusions:** Both pulmonary and hepatic CSE expression and H<sub>2</sub>S production decline in experimental HPS after CBDL, an effect not seen in non-cirrhotic portal hypertension where HPS does not develop. These findings indicate that the dramatic changes in H<sub>2</sub>S production in experimental cirrhosis are not due to the development of portal hypertension and are not confined to the liver. Exploring the mechanisms and consequences of reduced H<sub>2</sub>S production in cirrhosis and the possible role in experimental HPS may provide potential new therapeutic strategies.

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### 831

#### POST-TRANSLATIONAL REGULATION OF SINUSOIDAL ENDOTHELIAL CELL ENOS BY THE GPCR INTERACTOR, GIT1

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After liver injury, endothelial nitric oxide synthase (eNOS) activity is reduced in sinusoidal endothelial cells (SECs) and appears to result in reduction of nitric oxide (NO) synthesis, typical of portal hypertension. Previous studies of eNOS link post-translational abnormalities of Akt activation to GPCR signaling; therefore, we hypothesized that a GPCR kinase interacting protein (GIT1) might regulate eNOS activity. **METHODS:** SECs were isolated from normal or injured livers (BDL). eNOS, phospho-eNOS, and GIT1 expression were detected by immunoblotting. SECs were transduced with GIT1 cDNA and GIT1 siRNA. NO production was measured using the Griess reaction; a potential eNOS and GIT1 interaction was examined by immunoprecipitation. Adenovirus encoding GIT1 ( $1 \times 10^{10}$  pfu/kg) was transduced into SECs in vivo. **RESULTS:** Here, we report that GIT1 co-localizes and interacts with eNOS in SECs. GIT1 overexpression significantly increased NO production and enhanced Ser 1177 phosphorylation and Thr495 dephosphorylation without affecting total eNOS expression. The GIT1 and eNOS