In Vivo Longitudinal Measurements Of Brain Energy Metabolism in Chronic Hepatic Encephalopathy in a Rat Model using 31P MRS and 1H MRS

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TARGET AUDIENCE: Researchers involved in Hepatic Encephalopathy, Brain Energy Metabolism, ^{31}P MRS, Animal Models.

PURPOSE: Hepatic encephalopathy (HE) due to chronic liver disease (CLD) is a serious neuropsychiatric disorder. Patients suffer from an array of cognitive and fine motor deficits what highly affects the quality of life [1,2]. High ammonium (NH_4^+) delivery to the brain is causing accumulation of glutamine (Gln) in astrocytes and subsequent gradual release of other osmolytes (myoInositol (mIns), taurine (Tau), choline (Cho), a.o.) as a compensatory effect. In spite of this apparent osmoregulation, low grade brain edema is present as demonstrated by astrocyte swelling and ADC-apparent diffusion coefficients [3,4] and so far the mechanisms are not clear. Whether brain energy metabolism is affected is of some debate [5]. Although the energy metabolism disturbances in HE were proposed for the first time already in 1955 [6], there is no uniform answer to this question up to now. The aim of this study was to measure *in vivo* and longitudinally brain metabolism by ^{1}H MRS together with energy metabolism measured by ^{31}P MRS in animal model of CLD induced HE.

METHODS: To study CLD, we used bile duct ligated (BDL) rats, recognized by ISHEN (International Society for HE and Nitrogen metabolism) [7]. 7 adult Wistar rats were BDL and scanned before BDL and 4, 6 and 8 weeks after surgery. MR experiments were performed on a 9.4T system (Varian/Magnex Scientific). For ^{1}H MRS, we used home-built 14mm diameter quadrature ^{1}H surface coil as a transceiver, ultra-short-echo time SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 160 averages) [8], VOI of 2×2.8×2mm^3 localized in the hippocampus, due to its role in memory (a neurologic symptom in HE). First and second order shims were adjusted using FASTMAP (linewidth of 9-11Hz). Concentrations of metabolites were calculated by LCModel using water as internal reference. For ^{31}P MRS, we used home-built dual transceive surface coil with 17mm diameter quadrature ^{1}H loops and 15mm diameter single ^{31}P loop under proton ones, non-selective adiabatic half passage pulse [9] with 640 averages, OVS in x-, y- and z-direction and one dimensional ISIS in y for localization, VOI 4x7.5x6.5mm^3, shimming procedure the same as for ^{1}H. ^{31}P MR spectra were quantified using jMRUI software and phosphocreatine (PCr) concentration measured in the same VOI by ^{1}H MRS was used as a concentration reference. Plasma measurements of bilirubine and NH_4^+ and behavioral tests (10min Open field test + 5min Open field Novel object, to test motor activity and anxiety) were performed together with MR scans to proof the presence of HE [10].

RESULTS: Plasma measurements showed increased NH_4^+ (109±37μM versus 52±8μM in sham) and increased bilirubine levels (114.7±20μM versus <8.5μM in sham) 8 weeks after BDL. In behavioral tests, there was a significant difference in motor activity and anxiety between shams and BDL rats already 6 weeks after BDL surgery. From ^{1}H MRS from hippocampus at 8 weeks after BDL, we could see 2-fold increase of brain Gln ($p<0.0001$) and decrease in other brain osmolytes as a compensatory effect (mIns (-13%, $p<0.0001$), Tau (-6%, $p<0.05$)). We could measure also decrease in Cr (-7%, $p<0.05$), a metabolite involved in energy metabolism but recently described in osmoregulation and neuromodulation. ^{31}P MRS data showed a gradual non-significative 10% decrease of γ-ATP after rescaling to corresponding PCr concentration from ^{1}H MR spectra from the same VOI.

DISCUSSION AND CONCLUSION: Our present work demonstrates that the osmotic imbalance created by the continuous increase of Gln may be partially compensated by a concomitant decrease of other idiogenic osmolytes what is in agreement with previous measurements. However, in our previous studies ADC (apparent diffusion coefficient) values showed an increase (+10%) over the first 8 weeks post-BDL, suggesting that mild brain edema develops in spite of ongoing osmoregulation [3]. Our preliminary results with non-significative decrease in ATP suggest that residual brain edema is unlikely due to energy disturbances, what is in agreement with findings in ^{13}C MRS study with BDL rats [11]. High concentrations of the osmotically active Gln look, for the moment, as one of the principal causes for minimal brain edema. In the same time, it is worthy to note that 10% drop in ATP in living system is very important decrease, what more, our measurement represent a mean value of ATP from measured VOI, meaning that there can be regions in the brain affected even more and therefore further studies are needed.

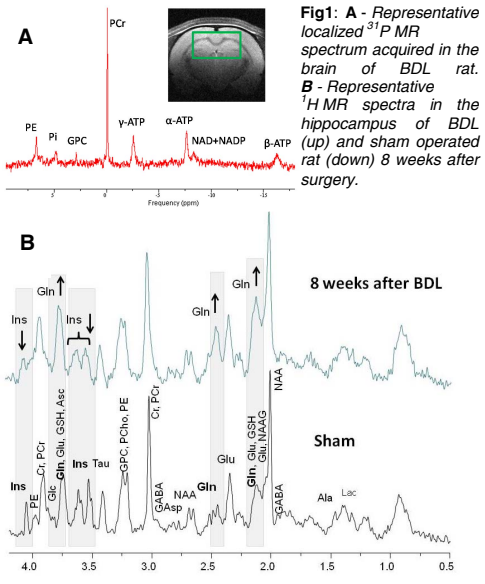

Fig1: **A** - *Representative localized ^{31}P MR spectrum acquired in the brain of BDL rat.* ***B*** *- Representative ^{1}H MR spectra in the hippocampus of BDL (up) and sham operated rat (down) 8 weeks after surgery.*

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