In-vivo ^{1}H MRS and ex-vivo ESR spectroscopy of Oxidative Stress in a rat model of Chronic Hepatic Encephalopathy

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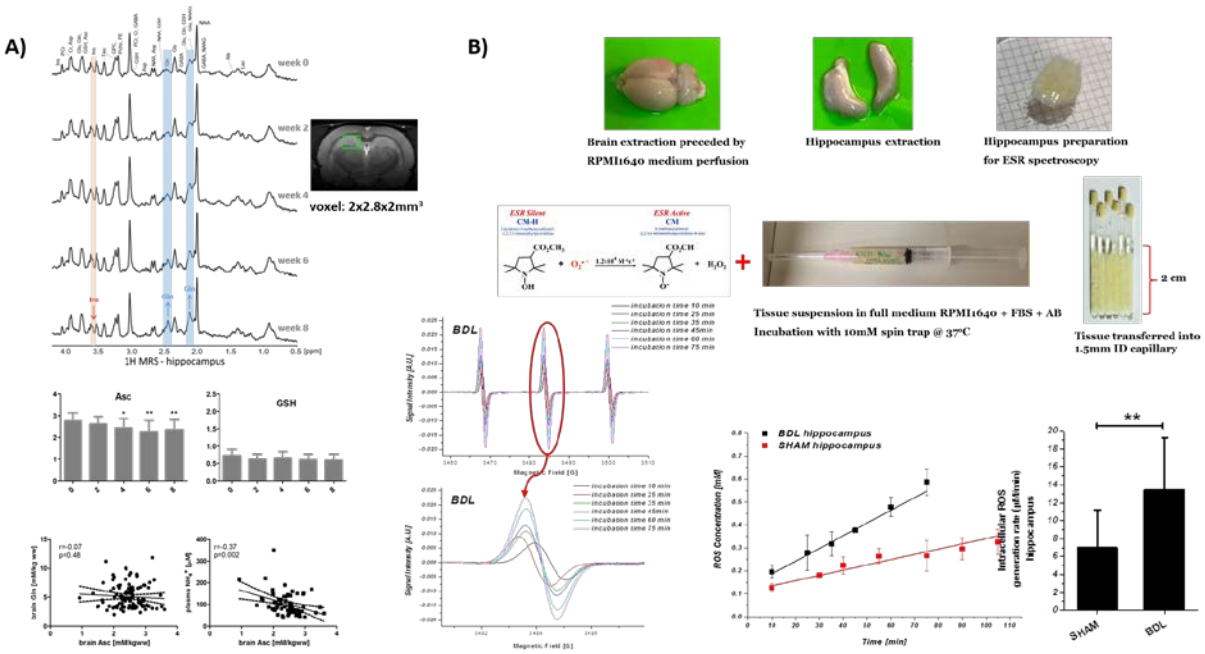
Introduction: Reactive oxygen species (ROS) are messenger molecules-components of signal transduction during physiological processes. In excess they lead to cellular dysfunction and are involved in neurotoxicity. The redox homeostasis is maintained by balance between ROS generation and elimination by antioxidants. Oxidative stress (OS) is believed to play a role in pathogenesis of chronic hepatic encephalopathy (CHE).CHE is accepted to occur from glutamine (Gln) accumulation secondary to NH_4^+ clearance impaired by the diseased liver. Impaired brain NH_4^+ detoxification with Gln increase seem to induce ROS generation. To date there are no *in-vivo* studies to assess the course of cerebral OS in CHE. The aim of this study was to investigate the OS in the hippocampus of a bile-duct ligated (BDL) rat model of CHE using *in-vivo* ^{1}H-MRS and *ex-vivo* ESR. **Methods:** ^{1}H-MRS: 9.4T-MR-system (Varian/Magnex-Scientific) using SPECIAL sequence (TE=2.8ms, TR=4sec; 160av). The hippocampus was scanned before BDL and after every 2 weeks up to week 8(n=20) **(A)**. Metabolites concentrations were calculated by LCModel. ESR: ESP300E spectrometer (Bruker BioSpin) with a TE_{102} cavity. The hippocampus was extracted at 6weeks post-BDL and sham-surgery (n=6), weighed, sliced and transferred into whole RPMI1640 medium with 10mM CMH spin trap (Noxygen). After each incubation (37°C) time tissue suspension was transferred into capillary tube and ESR signal was collected **(B)**.



Results/Discussion: Chronic liver disease was confirmed by the early increase in plasma NH_4^+ and bilirubin (2weeks post-BDL). The neurochemical pattern of CHE was observed: increase of Gln (4week post-BDL), decrease in main osmolytes (6week post-BDL) as an answer to Gln accumulation (Ins-30%,tCho-26%,Tau-8%,Cr-11%) and neurotransmitters decrease (Glu-11%) **(A).** Ins acts as a multifaceted player in cell signalling, influences the Ca^{2+} pump and can imbalance the ROS homeostasis. Asc showed a significant decrease at week4 compared to week0 (-12%,$p<0.05$) and reached -15% at week8 ($p<0.01$). Asc decrease over time correlated with plasma NH_4^+ increase ($r=-0.37$,$p=0.002$) but no correlation was observed with brain Gln **(A),** suggesting that the two may be directly linked in the pathogenesis of OS in rats with CHE. In addition, CMH intracellular $O_2^{\cdot-}$selective cell permeable spin probe was applied to evaluate ROS levels in hippocampus *ex-vivo*. Significant increase of hippocampal ROS levels in BDL ($p<0.01$,**B**) corroborates the ^{1}H-MRS findings of decreasing Asc **(A). Conclusion:** The present study shows the potential to monitor OS in a rat model of CHE using *in-vivo* ^{1}H-MRS combined with *ex-vivo* ESR. We detected, for the first time *in-vivo*, early changes in brain antioxidants, concomitant with Gln and NH_4^+ changes. These results were confirmed by ESR spectroscopy suggesting that central OS is an early event in CHE.

Acknowledgements: Supported by CIBM of the UNIL, UNIGE,HUG,CHUV, EPFL,the Leenaards and Jeantet Foundations and the SNSF project no 310030_173222/1.