Multimodal assessment of brain energy metabolism in a rat model of hepatic encephalopathy using ^{1}H-MRS and ^{18}F-FDG PET – a pilot study

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Synopsis

Hepatic encephalopathy (HE) is a severe complication of chronic liver disease which drastically affects patient lives. Its underlying mechanisms are still unknown and energy metabolism studies are of key interest. Here, we combined ^{18}F-FDG PET and ^{1}H-MRS and found a 2-fold decrease in brain glucose uptake in a rat model of HE compared to SHAM rats, associated with a previously reported increase in brain glutamine and decrease in osmolytes. Although the difference in glucose uptake measured by PET results from a combination of brain and systemic effects, this finding provides a new perspective on HE pathophysiology.

Introduction

Hepatic encephalopathy (HE) is a severe complication of chronic liver disease and leads to metabolic dysfunction and cognitive impairment, which are often lethal. The underlying mechanisms leading to this condition are still debated but the main focus so far has been on glutamine (Gln) metabolism. It is widely acknowledged[1] that chronic liver disease induces an excess of blood ammonia, which is not properly excreted and causes an increase in Gln in the brain. An increased brain Gln content is responsible for the decrease of the main osmolytes (Ins, Tau, tCho, tCr) through an osmotic regulation mechanism which possibly results in brain oedema[2]. However, very few studies on HE focused on energy metabolism so far. None of them showed significant changes in energy metabolites, neither for glucose steady state concentrations with ^{1}H-MRS[2] or metabolic fluxes with ^{13}C-MRS[3], nor for γ-ATP with ^{31}P-MRS[4]. Here we report a pioneering study on energy metabolism and glucose uptake by ^{18}F-FDG PET in a rat model of HE, combined with ^{1}H-MRS for disease assessment.

Methods

BDL rats - The bile duct ligated (BDL) rat model for chronic liver disease leading to HE[5] was used. Surgery was performed on adult male Wistar rats (n=12). Plasma bilirubin and ammonium were measured longitudinally. For all experiments, rats were under isoflurane anaesthesia (1.5-2%) and body temperature and breathing rate were monitored.
^{1}H-MRS - ^{1}H-MRS experiments on BDL rats were performed on an actively shielded 9.4T scanner, using SPECIAL[6] sequence (TE=2.8ms, TR=4s, 160 averages). Acquisitions were performed at weeks 0, 4 and 6 post surgery on two brain regions, hippocampus (week 0: n=4, week 6: n=9) and cerebellum (week 0: n=3, week 6: n=4), with a voxel size of 2x2.8x2 mm^3 and 2.5x2.5x2.5 mm^3, respectively. Absolute quantification of metabolites was performed with LCModel using the water signal as internal reference.
PET - PET experiments on BDL (n=10) and SHAM (n=8) rats were conducted after ^{1}H-MRS experiments at week 6, on an avalanche photodiode detector-based LabPET 4 scanner. After injection of a bolus of ^{18}F-FDG in the tail vein of the rat, a 45-min dynamic acquisition was performed on the region of the vena cava to extract the arterial input function (AIF)[7]. A 15-min static acquisition was then performed on the brain and a 3D map of the glucose cerebral metabolic rate (CMR_{glc}) was reconstructed using the Sokoloff method[8]. MLEM algorithm with the LabPET-4 built-in calibration tool was used to obtain quantified PET images in Bq/mL (1x1x1.18 mm^3 nominal resolution), corrected for radioactivity decay. The Lumped Constant (LC) was set to 0.71[7] and glycaemia values were assumed to be constant over the time of the experiment and were measured for each rat after the static acquisition. Averages of CMR_{glc} maps over spectroscopic voxels were computed for comparison with ^{1}H-MRS.

Results

Plasma bilirubin (<0.5 at week 0 (n=1) to 8.1mg/dl at week 6 (n=10)) and ammonia (26+/-7 at week 0 (n=8) to 60+/-30µM at week 6 (n=4)) increased in all BDL rats, confirming the induced chronic liver disease. The ^{1}H-MRS data confirmed the previously reported increase in brain glutamine[2] in both regions (fig 2), yet stronger in the cerebellum than in the hippocampus[9] (152% versus 73%). As expected, this increase was compensated by a significant decrease in the main osmolytes (Ins, Tau, tCr) (-15, -19%). The FDG input function was reliably measured from the dynamic PET images over the vena cava (fig 3A). The Sokoloff method, using the area under the curve of the FDG input function, LC, the steady-state brain image and glycaemia as inputs enabled the construction of high resolution metabolic maps (fig 3B). A typically 2-fold higher CMR_{glc} was observed in SHAM versus BDL rats on all coronal slices. PET-MRI coregistration allowed a quantitative comparison between PET and ^{1}H-MRS data (fig 4). A significant 2-fold decrease of CMR_{glc} in BDL rats compared to SHAM rats was measured in both brain regions, associated with a significant 2-fold increase in brain glutamine and 1.2-fold decrease in brain osmolytes in BDL rats at week 6.

Discussion

While ^{1}H-MRS provided a steady state information on metabolic pools in the hippocampus and the cerebellum, ^{18}F-FDG PET gave an additional and complementary information on glucose uptake. Because ^{18}F-FDG is converted to FDG-6P and no further degraded through the glycolysis, the PET signal at steady-state results from an accumulation of FDG-6P. It should be noted that the difference observed in CMR_{glc} is due to combined systemic and brain effects. Blood glycaemia values were significantly lower in BDL rats compared to SHAM rats. Yet, the difference measured between the two groups for CMR_{glc} maps (120%) was lower than the difference measured between glycaemia values (179%), suggesting an effect not restricted to systemic differences. Future blood glucose clamp PET studies are planned to separate the contribution of systemic and local metabolic changes on the CMR_{glc} of BDL rats.

Conclusion

A 2-fold decrease in glucose metabolic fluxes was observed in the hippocampus and cerebellum of BDL rats versus SHAM using ^{18}F-FDG PET. This finding correlates with an increase in glutamine and a decrease in osmolytes in both brain regions of BDL rats and paves the way for new insights on glucose function in the pathophysiology of HE.

Acknowledgements

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Figures

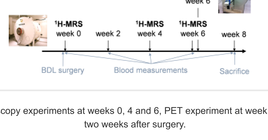

Figure 1 - Experimental plan: proton spectroscopy experiments at weeks 0, 4 and 6, PET experiment at week 6 and longitudinal blood measurements every two weeks after surgery.

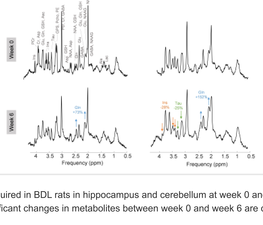

Figure 2 - Representative ^{1}H-MR spectra acquired in BDL rats in hippocampus and cerebellum at week 0 and week 6 post surgery. For each region, visual and significant changes in metabolites between week 0 and week 6 are displayed.

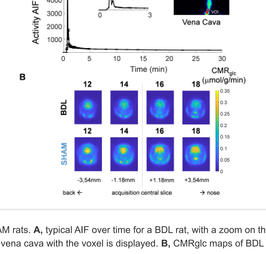

Figure 3 - PET experiments on BDL and SHAM rats. **A,** typical AIF over time for a BDL rat, with a zoom on the first 3 minutes after the bolus injection. The maximum intensity projection of the vena cava with the voxel is displayed. **B,** CMRglc maps of BDL and SHAM rats on 4 coronal slices.

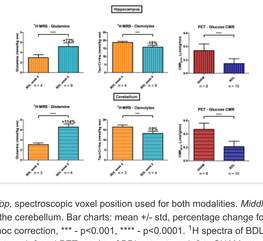

Figure 4 - ^{1}H-MRS – PET comparison. *Top,* spectroscopic voxel position used for both modalities. *Middle,* comparison of ^{1}H-MRS and PET in the hippocampus. *Bottom,* same comparison in the cerebellum. Bar charts: mean +/- std, percentage change for metabolites between week 0 and 6 in each region, 2-way ANOVA with Bonferroni post-hoc correction, *** - p<0.001, **** - p<0.0001. ^{1}H spectra of BDL rats at week 6 were compared to spectra of BDL rats at week 0 and PET results of BDL rats at week 6 to SHAM rats at week 6.