Chronic Hepatic Encephalopathy in the developing and adult rat brain: an in vivo non-invasive and longitudinal metabolic investigation using 1H MRS, DTI and immunohistochemistry

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Target Audience: researchers involved in the study of hepatic encephalopathy, brain metabolism, edema and different animal models

Purpose: Chronic liver disease (CLD) affects both adults and children and is often associated with some degree of hepatic encephalopathy (HE), a severe neuropsychiatric disorder with a high impact on quality of life [1-4]. Increase in ammonia delivery to the brain in CLD is thought to be the main culprit in HE [1-4]. In childhood, acute hyperammonemia (HA) is associated with brain edema and leads to irreversible damage of the developing central nervous system (CNS). Recent studies in infants and small children with chronic liver disease suggest that there is a neuropsychological deficit prior to transplant, suggesting that CNS changes occur early, and probably persist in these children. Long-term deficits of such a magnitude have not been described in adults with HA. Although cognitive deficits exist in children with CLD, the underlying mechanism is unclear [2]. How the developing brain responds to the metabolic changes of CLD, and how these mechanisms differ from those in adult patients are two unknowns. We hypothesized that blood-brain-barrier permeability and energy metabolism may be different in adult and developing brain.

Methods: Bile duct ligation (BDL) in rats is a frequently used animal model in CLD [5-7].Wistar adult and pup (21 days) rats were bile duct ligated and scanned before and weekly (up to 8 weeks) after BDL. Pup-age was chosen based on previous studies, which showed that the brain of a rat pup between P21-P30 corresponds to childhood. Experiments were performed on a 9.4T system (Varian/Magnex Scientific) using a home-built 14 mm diameter quadrature ^{1}H coil as a transceiver. The ultra-short-echo time SPECIAL spectroscopy sequence (TE=2.8ms, TR=4s, 160 scans) [8] was used to localize a VOI of 2×2.8×2mm^3 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 9-12Hz). Concentrations of metabolites were calculated by LCModel using water as internal reference. Diffusion tensor acquisitions were done with double spin echo semi-adiabatic 4 shots EPI sequence [9] (FOV: 23 × 15 mm^2, Acq matrix 128 × 64, 6 slices, 1 mm thk, 8 averages, TE/TR = 42/2000 ms). Diffusion gradients (Gdiff = 19.7 G/cm, δ = 3 ms, Δ = 20 ms, giving a b-value of 1079 s.mm^{-2}) were applied along 21 directions. Diffusivity values (ADC-apparent diffusion coefficient, FA-fractional anisotropy) were derived from the tensor using a Matlab (Mathworks, Natick, MA) script. ADC was measured in ROIs positioned in: cortex, striatum and hippocampus.Immunohistochemistry on brain tissue was performed using astrocytic and water channel markers (GFAP, AQP4).

Results and Discussion:

Following BDL, brain metabolism differed in pups and adults (Table 1). Changes in pups were more significant for all parameters studied. ^{1}H spectra exhibited excellent SNR allowing easy separation of Gln from Glu. Notable differences in metabolite signals were apparent already from the spectra (i.e. increase Gln, decrease Ins, Tau).

Table 1 Brain metabolism in pups and adults following BDL.

	Pups (8 weeks after BDL)	Adult (8 weeks after BDL)	p-value
Glutamine (Gln)	↑ ~300%	↑ ~200%	<0.001
Myo-inositol (Ins)	↓ ~50%	↓ ~30%	<0.001
ADC	↑ ~30%	↑ ~10%	<0.05
Glutamate (Glu)	↓ ~20%	↓ ~10%	<0.01
Aspartate (Asp)	↓ ~50%	↓ ~30%	<0.01
Glutathione (GSH)	↓ ~36%	↓ ~9%	<0.01 (pups)
Lactate (Lac)	↑~30%	↑~10%	<0.05 (pups)
Immunohistochemistry:			
GFAP expression	Astrocyte swelling	Astrocyte swelling	
AQP4 expression	↑↑	↑	

First, pups displayed a more pronounced increase of brain Gln than adults after BDL, reaching a threefold increase, with a more pronounced decrease in the osmolyte Ins (~50%). Assessment of brain edema showed, at 8 weeks after BDL, significantly higher ADC values in BDL pups (~30%) compared with adult BDL rats (~10%), suggesting that low grade edema is noticeable in spite of ongoing osmoregulation (decreased Ins). Developing and adult BDL rats present typical swelled astrocytes by GFAP and increase of AQP4 expression in microcapillary endothelial cells, in particular for pups. Enhanced expression of AQP4 in microcapillary endothelial cells, in particular for pups suggest that the upregulation of this water channel might contribute to increase BBB permeability and might be part of mechanism underlying the higher ADC values in pups compared with adults.

Second, pups showed a greater decrease in brain neurotransmitters and antioxidants (Glu, Asp and GSH) coupled with an increase in energy metabolites (i.e. 30% increase in Lac compared with 10% in adults). The underlying mechanistic hypothesis here is that NH4+ may have a more pronounced effect on the mitochondrial permeability transition during brain development than in adults.

We conclude that osmotic and metabolic changes are greater in pups than adults, possibly owing to increased BBB permeability. How these two processes are linked and contribute to edema and neuro-cognitive changes remains to be determined. Furthermore, these data show novel metabolic changes potentially associated with CLD and offering novel insight into the metabolic anomalies underlying HE in both adult and developing brain. Finally, our technics allow investigating in vivo and simultaneously the chronological involvement of metabolic events and edema during the progression of HE, something never studied.

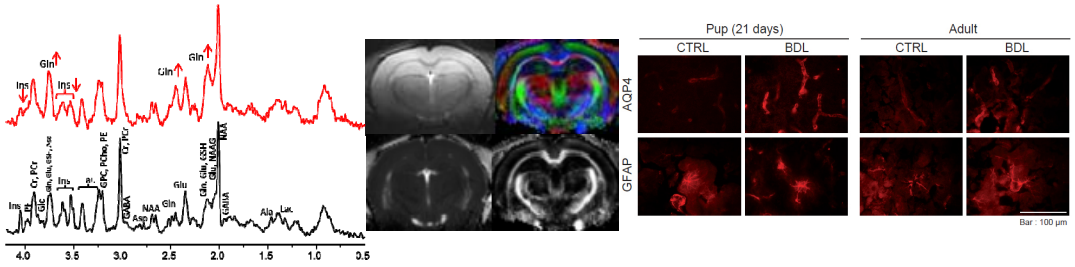

Fig. 1: Left: Representative ^{1}H MRS spectra in the hippocampus of a control (up) and BDL (bottom) adult rat; Middle: Images acquired with the semi-adiabatic SE-EPI before BDL. From left to right: B_0 image (b = 0 s.mm^{-2}), color encoded map, diffusion tensor trace (ADC) and fractional anistropy (FA) map; Right: Expression of aquaporine 4 (AQP4) and glial fibrillary acidic protein (GFAP) under BDL conditions, in the cerebral cortex of pups (21 days) and adult rats.

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