Neuroprotective effect of Lactoferrin following inflammatory injury in the developing rat brain assessed by high-field ^{1}H-MR Spectroscopy

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Target audience: Inflammatory models of perinatal brain injury, Neuroprotection by Lactoferrin, ^{1}H-Magnetic Resonance Spectroscopy.

Introduction: The 3-day old rat (P3) shares some similarities in terms of cortical neuronal, glial and oligodendroglial development to the very preterm infant around 24-28 weeks of gestation. One main cause of brain lesion in the early preterm is infection induced inflammation. As such, animal models of inflammatory preterm brain injury can be achieved by bacteria-derived lipopolysaccharide (LPS) exposure[1]. Lactoferrin (Lf) is an iron-binding glycoprotein secreted in milk known as antioxidant, antimicrobial and anti-inflammatory[2,3]. The aim of this work was to assess the neuroprotective effect of Lf in the very immature LPS model. For this purpose a multimodal NMR protocol was performed at 9.4T: T_2W imaging and ^{1}H-Magnetic Resonance Spectroscopy (MRS).

Materials and Methods: Dams received either Lf-enriched food (0.85% Lf, 1 g/kg/day) or a diet isocaloric (Iso) to the Lf from the birth of pups (P0) and during 3 weeks. Pups were then divided in four groups: Sham-Iso, LPS-Iso, Sham-Lf and LPS-Lf (n=10/group). Therefore, rat pups received Lf through breastfeeding. At P3 pups from LPS-Lf and LPS-Iso groups were anesthetized with isoflurane and injected with 0.5 µL saline containing LPS (10µg) in the subcortical white matter. Sham groups received a vehicle injection in similar conditions. Effect of Lf was assessed at two different time points: 1 and 20 day(s) following LPS injection. MR experiments were performed on an actively-shielded 9.4T/31cm magnet (Agilent/Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs) with a quadrature transceive 20-mm surface RF coil. At P4 and P24: Fast Spin Echo T_2W images were performed to position MRS voxel of interest (VOI) and to quantify the volume of the ventricles. After automatic FASTMAP[4] shimming, spectra acquisition was performed on two different brain regions: hippocampus (Hp, VOI=1×2×2.5 mm^3) as well as striatum (St, VOI=2×2×2 mm^3) within the injected hemisphere using an ultra-short echo time (TE/TR = 2.7/4000 ms) SPECIAL spectroscopy method[5] and analyzed with LCModel[6]. Volumes of ventricles were quantified using BrainVisa/Anatomist[7]. A Mann-Whitney test was used to compare statistically values between the different groups (significance for P<0.05).

Results: Not any difference (MRI and MRS) was observed between Sham-Iso and Sham-Lf rats; as such the 2 groups were pooled into only one Sham group.

MRI (Fig. 1A): at P4, not any change in ventricular size was observed. At P24 both LPS exposed groups presented significant ventriculomegaly, with a tendency to smaller ventricle volumes in the LPS-Lf (25±2mm^3) than in the LPS-Iso group (34±3mm^3).

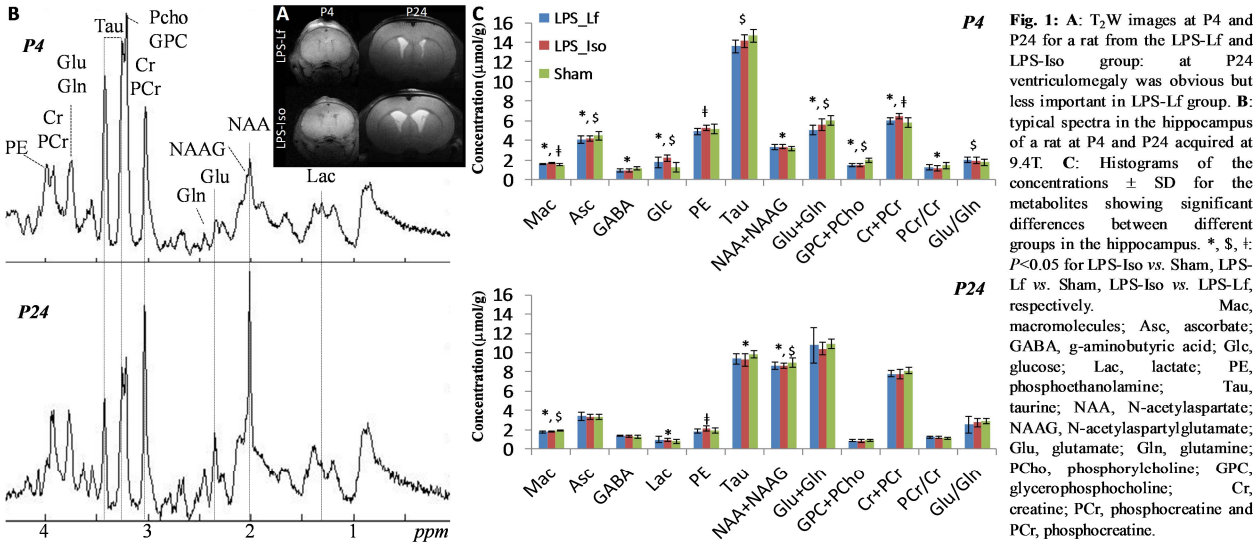

Fig. 1: A: T_2W images at P4 and P24 for a rat from the LPS-Lf and LPS-Iso group: at P24 ventriculomegaly was obvious but less important in LPS-Lf group. **B**: typical spectra in the hippocampus of a rat at P4 and P24 acquired at 9.4T. **C**: Histograms of the concentrations ± SD for the metabolites showing significant differences between different groups in the hippocampus. *, $, ǂ: P<0.05 for LPS-Iso *vs.* Sham, LPS-Lf *vs.* Sham, LPS-Iso *vs.* LPS-Lf, respectively. Mac, macromolecules; Asc, ascorbate; GABA, g-aminobutyric acid; Glc, glucose; Lac, lactate; PE, phosphoethanolamine; Tau, taurine; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; Glu, glutamate; Gln, glutamine; PCho, phosphorylcholine; GPC, glycerophosphocholine; Cr, creatine; PCr, phosphocreatine and PCr, phosphocreatine.

MRS (Fig. 1B and C): very good spectral quality was achieved in the current study (SNR=19±4 - Fig. 1B). For clarity, only metabolic changes in the Hp are presented but changes in the St were comparable. At 24h, LPS exposed groups (*i.e.* -Lf and -Iso) exhibited altered metabolism (Fig. 1C) compared to Sham group involving modification of [Glc]-energy source, [Glu+Gln]-involved in neurotransmission and in the Glu-Gln cycle between neurons and glia and [GPC+PCho]-components of cell membranes. LPS-Iso group presented in addition changes in [Mac]-putative tissue integrity marker, [GABA]-primary inhibitory neurotransmitter, [NAA+NAAG]-marker of neuronal damage/suffering and [PCr]/[Cr]-marker of the energetic status of the cells; compared to Sham group. Finally LPS-Iso group presented also differences with the LPS-Lf group: [Mac], [PE]-components of cell membranes and [Cr+PCr]-implicated in energetic metabolism. At long term (P24-Fig. 1C) metabolism was less disturbed. Both LPS exposed groups (*i.e.* -Lf and -Iso) showed changes in [Mac] and [NAA+NAAG] compared to Sham group. LPS-Iso rats presented in addition increase of [Lac]-marker of anaerobic metabolism and found in necrotic tissue and decrease of [Tau]-implicated in neurotransmission; compared to Sham rats. Finally, [PE] was increased in the LPS-Iso group compared to LPS-Lf group.

Discussion and conclusion: This study shows acute and long-term characterization of inflammatory injury in the P3 developing brain and the neuroprotective effect of Lf using MRS/MRI. LPS cerebral exposure leads to acute changes in neurochemical profile at 24h post-injection including altered neurotransmission, energetic metabolism and cell/tissue integrity. The Lf supplemented in food during lactation reduces LPS-induced alteration of the neurochemical profile at 24h. In addition, some metabolites were also different between the LPS-Iso and LPS-Lf group. In a lesser extent, the metabolism of LPS exposed P24 rats continued to be disturbed with changes of some metabolites known as tissue integrity markers and some involved in neurotransmission. As at P4, LPS-Lf rats presented less compromised metabolism than LPS-Iso rats. To conclude, Lf supplemented in the food during the lactation appears to have a neuroprotective effect with reduced alterations in Hp/St neurochemical profiles and in LPS induced ventriculomegaly for the LPS-Lf animals. Future work will assessed the effect on microstructure by DTI as well as histopathology to better understand mechanisms of neuroprotection. This result could be of high interest in the clinical field of preterm's brain neuroprotection.

References: 1. Kumral A. Neonatology 2007; 2. Somm E. Pediatric Research In press; 3. van de Looij Y. ISMRM 2012; 4. Gruetter R. MRM 2000; 5. Mlynárik V. MRM 2006; 6. Provencher SW. MRM 1999; 7. Rivière D. Neuroimage 2003. **The two first authors contributed equally to this work.**

Supported by the Fond National Suisse (N° 31003A-135581/1), the CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL, Leenards and Jeantet foundation.