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Dose-dependent neuroprotective effects of Bovine Lactoferrin following neonatal hypoxia-ischemia in the immature rat brain

Yohan van de Looij[1,2], Eduardo Sanches[1], Sadou Saw[1], Audrey Toulotte[1], Analina Da Dilva[2], Laura Modernell[1], and Stéphane V Sizonenko[1]

[1]Division of Development and Growth, Department of Paediatrics and Gynaecology-Obstetrics, School of Medicine, University of Geneva, Geneva, Switzerland, [2]Center for Biomedical Imaging, Animal Imaging Technology section, Federal Institute of Technology of Lausanne, Lausanne, Switzerland

BACKGROUND

Injuries to the developing brain due to hypoxia–ischemia (HI) are common causes of neurological disabilities in preterm babies. HI, with oxygen deprivation to the brain or reduced cerebral blood perfusion due to birth asphyxia, often leads to severe brain damage and sequelae. Injury mechanisms include glutamate excitotoxicity, oxidative stress, blood brain barrier dysfunction and exacerbated inflammation. Nutritional intervention is emerging as a therapeutic alternative to prevent and rescue brain from HI injury. Lactoferrin (Lf) is an iron-binding protein present in saliva, tears and breast milk which has been shown to have antioxidant, anti-inflammatory and anti-apoptotic properties when administered to mothers as a dietary supplement during pregnancy and/or lactation in preclinical studies of developmental brain injuries. However, despite Lf's promising neuroprotective effects, there is no established dose [1].

AIMS

The aim of this work was to test three different doses of dietary maternal Lf supplementation using the postnatal day 3 HI model and evaluated the acute neurochemical damage profile using ^{1}H Magnetic Resonance Spectroscopy (MRS) and long-term microstructure alterations using advanced diffusion imaging (DTI/NODDI) allied to protein expression and histological analysis.

METHODS

Pregnant Wistar rats were fed either control diet or bovine Lf supplemented at 0.1, 1 or 10 g/kg/body weight concentration from the last day of pregnancy (E21) to weaning.
P3: right carotid artery cauterization followed by 30 min at 6% O2.
MRS/MRI: actively-shielded 9.4T/31cm magnet (Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs)
MRS : quadrature transceive 20-mm surface coil. VOI of 1.5×1.5×2.5mm3 within the cortical lesion using an ultra-short echo time (TE = 2.7 ms) SPECIAL [2]. Proton spectra analyze : LCModel [3].
P25: Ex-vivo diff MRI with a 2.5 mm Ø birdcage coil. Multi-b-value shell protocol, SE sequence : 96 DWI: 15 b0 and 81 in 3 shells (# of directions/b-value in s/mm^2): 21/1750, 30/3400 and 30/5100.
Acquired data reconstructed with DTI-TK and fitted using the NODDI toolbox [4].
Three different brain regions identified: corpus callosum, cingulum and external capsule.

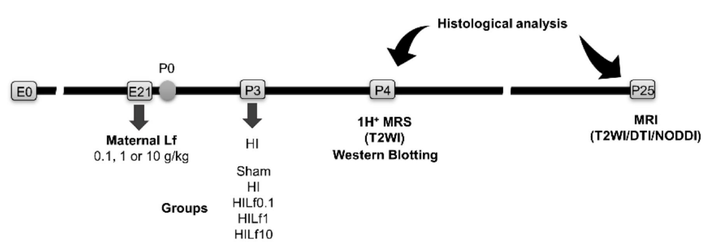

Figure 1: Experimental timeline of the study. E: Embryonic day, HI: Hypoxia-Ischemia, P: Postnatal Day, DTI : Diffusion Tensor Imaging, Lf: Lactoferrin, NODDI: Neurite orientation dispersion and density imaging.

RESULTS

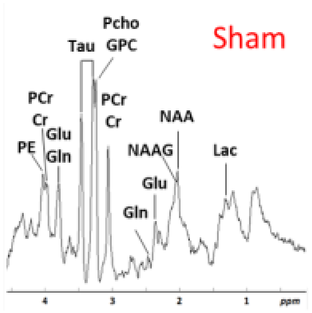

Figure 2: Typical cortical spectrum obtained in the Sham group at P4.

- ✓**Huge metabolic disturbance following HI**
- ✓**Dose dependent effect of Lf as neuroprotector**

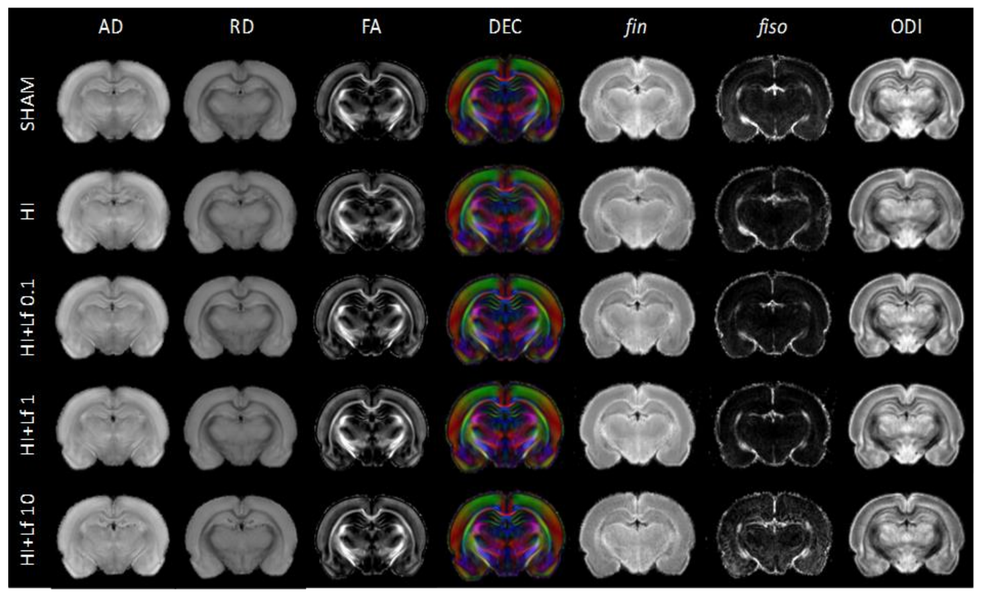

Figure 4: Diffusivity (Axial, AD and Radial, RD), fractional anisotropy (FA) and direction encoded color (DEC) maps, intra-neurite volume fraction (fin), cerebrospinal volume fraction (fiso), and orientation dispersion index (ODI) maps. Maps correspond to the averaged maps over each group.

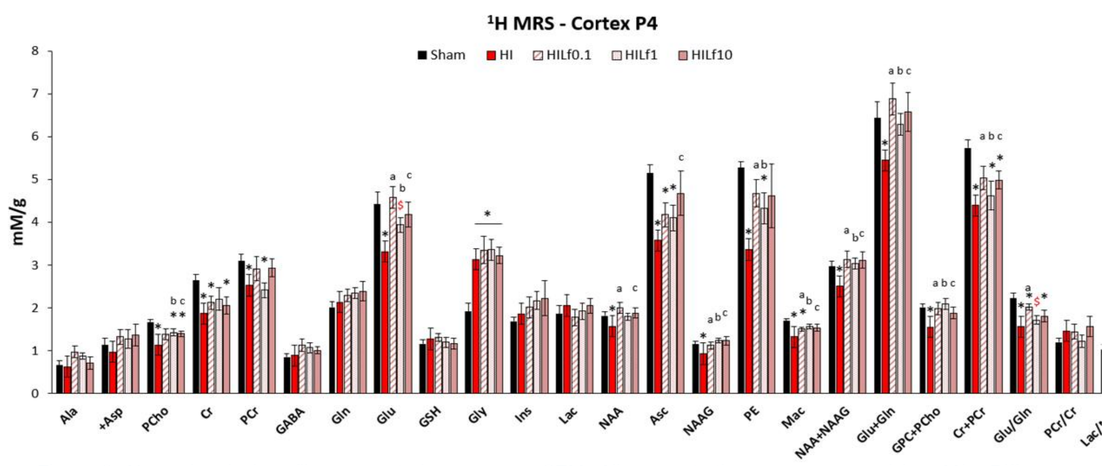

Figure 3: Neurochemical profiles presented as mean ± SEM. All concentrations are expressed in mM/g. Differences between groups (Sham, HI, HILf0.1, HILf1 and HILf10) 24h post-HI ($p<0.05$, * HI vs. SH, $ difference between HILf groups. a b c HILf 0.1, 1 or 10 vs HI respectively. Differences were determined by one-way ANOVA and considered significant when $p<0.05$

- ✓**WM defect long term following HI**
- ✓**0.1 and 1 mg/Kg neuroprotective doses**

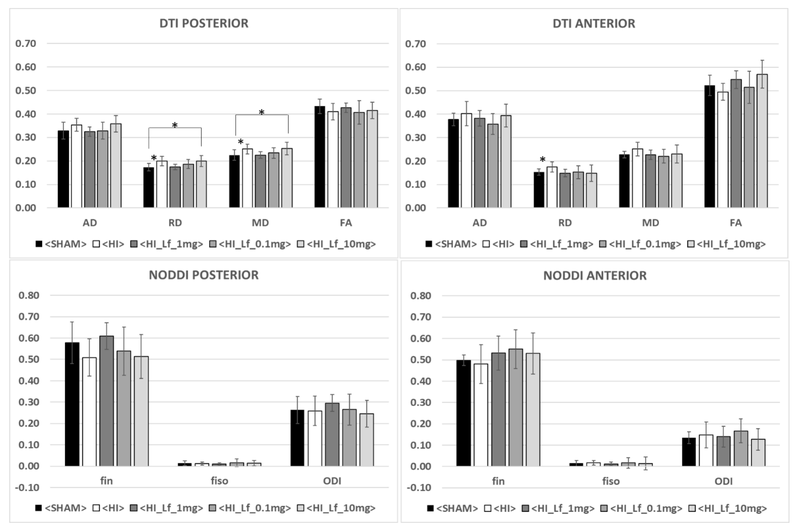

Figure 5: Histograms of the mean values of Diffusivity (Mean, MD; Axial, AD and Radial, RD), fractional anisotropy (FA), intra-neurite volume fraction (fin), isotropic volume fraction (fiso) and orientation dispersion index (ODI) for Sham, HI, HILf01, HILf1 and HILf10 rats. *: P<0.05.

CONCLUSION

In conclusion, Lf supplementation attenuates, in a dose-dependent manner, the acute and long-term cerebral injury caused by HI. This study suggests that Lf reached its optimal effects with dose of 1g/kg whereas 10mg seems deleterious for some aspects. Further investigations are in progress to better understand mechanisms of Lf.

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