Neuroprotective effect of lactoferrin following inflammatory injury in the developing rat brain assessed by high-field neurite orientation dispersion and density imaging

Yohan van de Looij[1,2], Vanessa Ginet[1], Petra S Hüppi[1], and Stéphane V Sizonenko[1]

[1]*Division of Child Growth and Development, University of Geneva, Geneva, Switzerland,* [2]*Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*

Target audience: Inflammatory model of perinatal brain injury, neuroprotection by lactoferrin, diffusion imaging (DTI and NODDI).

Introduction: One of the main causes 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] in the 3-day old rat (P3) sharing some similarities in terms of cortical neuronal, glial and oligodendroglial development to the very preterm infant around 24-28 weeks of gestation. Lactoferrin (Lf) is an iron-binding glycoprotein secreted in milk known as antioxidant, antimicrobial and anti-inflammatory[2]. In a previous work we showed on a model of intracerebral injection of LPS that Lf supplemented in food during lactation reduced LPS-induced alteration of the neurochemical profile at 24h as well as ventriculomegaly[1]. Recently, the neurite orientation dispersion and density imaging (NODDI[3]), a new practical diffusion MRI technique for estimating the microstructural complexity of neurites (*i.e.* dendrites and axons) has been successfully used *in vivo* on clinical MRI scanners[3]. NODDI provides estimation of microstructural parameters such as intra-neurite volume fraction (f_{icvf}), extra neurite-volume fraction (f_{ecvf}), cerebrospinal volume fraction (f_{iso}) and a new index called orientation dispersion index (ODI) to model the dispersion/fanning of the axonal fibers or dendrites. The aim of this work was to assess long-term neuroprotective effect of Lf on brain microstructure by using diffusion imaging and NODDI model at 9.4T in our model of LPS-induced inflammatory injury in the very immature brain.

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. Lf dosage in sucked milk and serum showed that rat pups received Lf through breastfeeding[4]. At P3 pups were anesthetized with isoflurane and injected with 1 µL of NaCl (Sham) or NaCl containing LPS (10µg) in the subcortical white matter. Three groups were studied: NaCl (Sham), LPS and LPS_Lf (n=14/group). Effect of Lf was assessed 20 days following LPS injection. MR experiments were performed on an actively-shielded 9.4T/31cm magnet (Agilent) equipped with 12-cm gradient coils (400mT/m, 120µs) with a quadrature transceive 20-mm surface RF coil. A multi-b-value shell DWI protocol was acquired using EPI 4-shots sequence with the following parameters: FOV = 23×15 mm^2, matrix size = 128×64, 8 slices of 0.8 mm thickness in the axial plane, 6 averages with TE/TR = 42/2000 ms. A total of 54 DWI were acquired, three of them were b_0 reference images. The remaining 51 were separated in 2 shells with the following distribution (# of directions/*b*-value in s/mm^2): 21/1000 and 30/2000. All 51 directions were non-collinear and uniformly distributed in each shell. The acquisition time was 2h. Acquired data were fitted using the NODDI toolbox[3]. Ventricle volumes were measured. A Mann-Whitney test was used to compare statistically values measured in the corpus callosum (CC) and striatum (St) between the different groups (significance for $P<0.05$).

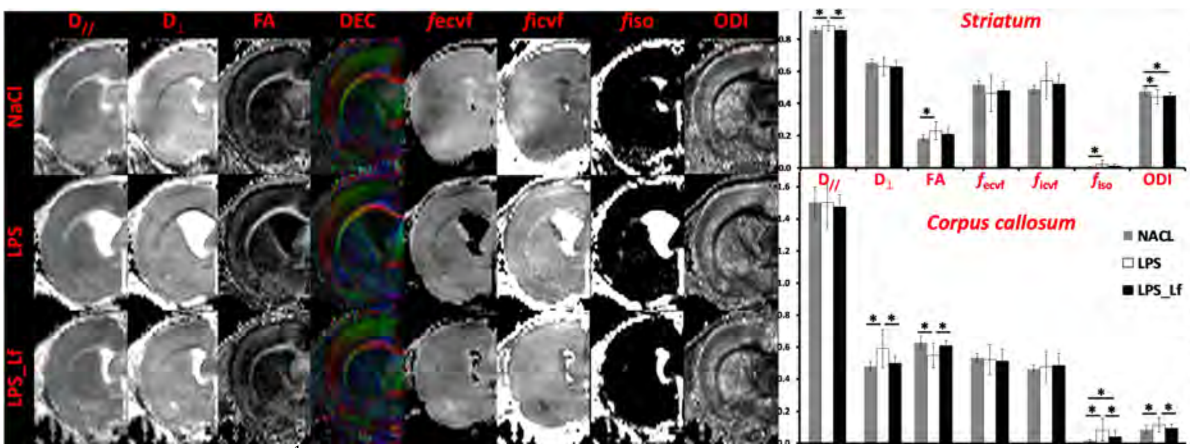

Fig. 1: DTI derived maps: diffusivity ($D_{//}$ and $D_{\perp}$), FA and color maps as well as NODDI derived maps, f_{ecvf}, f_{icvf}, f_{iso} and ODI maps of a typical ipsilateral NaCl, LPS and LPS_Lf rat brain. Right panel: mean values±SD of these parameters in the St and CC for each group. *: $P<0.05$, diffusivity: $\times 10^{-3} mm^2.s^{-1}$.

Results: As previously shown[1] LPS exposed groups presented obvious ventriculomegaly which was significantly reduced in Lf-treated rat pups (LPS: 24.18±3.32 mm^3; LPS_Lf: 12.87±3.06 mm^3; $P<0.05$). In the CC (Fig.1), a significant decrease of FA related to increase of $D_{\perp}$ was observed in the LPS group compared to both NaCl and LPS_Lf groups. Indeed, f_{iso} and ODI were increased in LPS group compared to both NaCl and LPS_Lf groups. In the St (Fig. 1), $D_{//}$ and FA were significantly increased in the LPS group compared to NaCl whereas restored in the LPS_Lf group; f_{iso} was increased in the LPS rats whereas ODI was decreased in the LPS and LPS_Lf rats compared to NaCl.

Discussion and conclusion: In this study we show for the first time feasibility of NODDI *in-vivo* on the rat brain at 9.4T. LPS cerebral exposure leads to acute changes in the CC: myelination defect as depicted by FA and $D_{\perp}$ changes; ODI might be increased by injured CC with fibers less compacted whereas f_{iso} increase is probably related to water contamination from ventricles. All these changes are restored in the CC of Lf-supplemented rats. In the St, microstructure becomes more organized (axonal fascicle area increases - data not shown) with LPS as depicted by increased $D_{//}$ and FA as well as decreased ODI. In the other hand, f_{iso} increases in LPS group and in a less extent in the LPS_Lf groups, probably related to water contamination from ventricles as in the white matter. A partial neuroprotection is observed in the St of Lf supplemented rats with only abnormal ODI values. **In conclusion**, Lf supplemented in food during lactation reduces long-term LPS-induced alterations of the microstructure. NODDI investigation is very relevant in such application, leading to very accurate assessment of LPS-induced brain injury and Lf neuroprotective effects.

References: 1. van de Looij Y. #3768 in ISMRM 2014; 2. Somm E. Ped. Res. 2014; 3. Zhang H. NeuroImage 2012; 4. van de Looij Y. Annals Clin. Trans. Neurol. 2014. **Supported by** the Fond National Suisse (N° 31003A-135581/1), the CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL, Leenards and Jeantet foundation.