Lactoferrine: Une protéine du lait pour protéger le cerveau en développement ?

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The making of the brain

Migration
- Organisation: Orientation of neurons, interconnections, synapses

Proliferation
- Subplate
- Radial Glia

Glial cell differentiation
- Myelination

Cortical folding

Weeks gestation
- 10
- 15
- 20
- 25
- 30
- 35
- 40

Prematurity
Encephalopathy of prematurity

CONSEQUENCES OF PREMATURE BIRTH ON BRAIN STRUCTURE AND FUNCTION

- Altered microstructure
- Smaller volumes of the cerebral cortex and white matter
- Reduced sulcal complexity
- Altered thickness of cerebral cortex
- Altered activation of cortical areas during resting state

Illustration L. Vasung
Encephalopathy of prematurity

Developmental modulators and injury of the brain

**Antenatal factors**
- hypoxia-ischemia
- inflammation, cytokines
- oxidative stress
- malnutrition
- maternal stress
- toxins

**Perinatal factors**
- hypoxia-ischemia
- oxidative stress
- inflammation, cytokines
- epi-genetic factors

**Postnatal factors**
- hypoxia-ischemia
- oxidative stress
- inflammation, cytokines
- loss of maternal GF
- pain, stress
- drugs
- nutrition
- epi-genetic factors
Lactoferrin and the developing brain

- Lactoferrin (LF) is a glycoprotein secreted in high concentration in milk
- Has **anti-infectious, anti-inflammatory and antioxidant** activities (*Garcia-Montoya IA et al., Biochimica et biophysica acta 2012*)
- After oral administration, LF is rapidly and actively transferred from the intestine into various organs including the brain (*Ji B, et al. Life Sci 2006*)
- LF oral supplement reduces anemia in pregnancy and preterm delivery through reduction of IL6 (*Paesano, Biochem Cell Biol 2012*) and in a rabbit model of inflammatory preterm delivery (*Hasegawa, A. Am J Obst & Gyn, 2005*)
- LF oral supplement has been shown to reduce late onset sepsis in the preterm infants (*Manzoni et al., JAMA: 2009*)

*Ochoa, T. and Sizonenko S.V., Biochemistry and Cell Biology, 2017*
**Lactoferrin**

**Protection**
- Downregulation of pro-inflammatory cytokines
- Secretion by activated microglia
- Binding of bacterial virulence factors
- Antiapoptotic
- Regulation of host immune response
- Iron toxicity reduction
- Oxidative stress reduction

**Support**
- Promotion of neurotrophic factors expression
- Support of brain development
- Neuronal differentiation factors expression

*Ochoa, T. and Sizonenko S.V., 2017, Biochemistry and Cell Biology*
To study the neuroprotective effect of maternal supplementation with Lf in animal models of developmental/preterm brain injury:

- Intra-uterine growth restriction (IUGR)
- Hypoxia-Ischemia (HI)
- Inflammation (LPS)
Advanced Magnetic Resonance imaging: a multi-modal tool to study brain development, injury and neuroprotection.
IUGR + Lf

- *In-utero* exposure to dexamethasone (Dex) mimics maternal stress and the clinical situation when glucocorticoids are administered to pregnant women at risk of premature delivery.

- The rat model of glucocorticoids exposure during fetal life has shown IUGR at birth and delay in cerebral maturation with neuronal and white matter alterations.

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**Somm et al, 2014 Pediatric Research**
IUGR + LF
Body weight

Somm et al, 2014 Pediatric Research
IUGR + LF
Hippocampus metabolism at P7

Somm et al, 2014 Pediatric Research
IUGR + LF
White matter at P21

Somm et al, 2014 Pediatric Research
IUGR + LF
Gene expression at P7

- In vivo DEX down regulates BDNF expression in hippocampus (Barbany G, Persson H. Eur J Neurosci 1992)
- In cortical cell culture models, chronic Dex exposure suppress BDNF-induced glutamate release (Numakawa T et al. PNAS 2009).
- DMT-1 is an iron transporter expressed in various brain cells

Somm et al, 2014 Pediatric Research
Hypoxia-Ischemia injury in the P3 rat + LF

P3 rat pup: Brain development equivalent to 24-28 weeks prterm infant

**LACTATION**

- P0 Lactoferrin supplementation in dam food (1g/kg/d) P25

**P3 HI**
- Hypoxia @ 6% O<sub>2</sub>
  - 0
  - 30
  - 60

Right carotid artery cauterization

**T<sub>2</sub> Imaging screening**

**P25: T2MRI / DTI / MRS**

1. **HI-Lf: HI injury + Lf** supplemented food to the dam starting on day of birth; n=16
2. **HI-Iso: HI injury + Lf free** isocaloric food to the dam starting on day of birth; n=15
3. **Sham: No HI injury**, Lf or isocaloric food; n=9 (animals were pooled as showed no difference in the analysed parameters)

*Van de Looij et al, Annals of Clinical and translational neurology, 2014*
Hypoxia-Ischemia at P3: alteration of microstructure

Fluoro-Jade B
Astrocytes
Radial glia
Microglia

S.V. Sizonenko et al. Cerebral Cortex 2007: 17:2609-17
Y. van de Looij, Magnetic resonance in Medicine 2011: 65(2)
Longterm consequences of HI at P3 on brain development

Volume reduction

Cortical white matter

Myelination

S.V. Sizonenko et al. Cerebral Cortex 2007: 17:2609-17
Y. van de Looij, Magnetic resonance in Medecine 2011: 65(2)
Long term alteration of white matter microstructure

Anisotropy (FA) ± SD

S.V. Sizonenko et al. Cerebral Cortex 2007
Y. van de Looij, MRM 2011
HI injury + Lf: volume of injury

HI injury + Lf: white matter damage

HI injury + Lf: white matter damage

HI injury + Lf: genes, proteins expression

**Inflammatory injury in the P3 rat + Lf**

**LACTATION**

- **P0** Lactoferrin supplementation in dam food
- **P3** LPS (10µg) or NaCl injection in the Corpus Callosum
- **P4** $T_{2}$WI/$^1$H-MRS, Histology
- **P7** Histology
- **P25** $T_{2}$WI/$^1$H-MRS

**3 Experimental groups:**

- **LPS-Lf**: Lf-enriched food (1 g/kg/day) *ad libitum* during lactation + LPS injury; n=14
- **LPS-Iso**: diet isocaloric to the Lf food *ad libitum* during lactation + LPS injury; n=14.
- **Controls**: Lf enriched or isocaloric food + NaCl injection; n=10.

*Ginet V. et al, Biofactors, 2016*
Inflammatory injury in the P3 rat + Lf

Ginet V. et al, Biofactors, 2016
Inflammatory injury in the P3 rat + Lf

Ginet V. et al, Biofactors, 2016

Figures:

(Figure 1): Lactoferrin reduced LPS-induced ventriculomegaly. (A) Typical T2*W images of ipsilateral rat brains from CT, LPS and LPS+Lf (lactoferrin) group 24h (P4) and 20 days (P24) after NaCl or LPS injection and the corresponding quantification of the mean ventricle volumes. (B) Representative cresyl violet stains of coronal brain sections and the corresponding ipsilateral ventricle volume quantification (expressed as a percentage of total brain volume). (C) Volume quantification of the cortex, the ipsilateral lateral ventricle, the...
Inflammatory injury in the P3 rat + Lf

Myelinated axons were cross-sectioned, stained with Nissl method, and the number of SMI-312 immuno-positive axonal fascicles was determined. NaCl and LPS were injected into the striatum, and Lactoferrin (Lf) was co-administered in a subset of animals. The number of fascicles was counted in three size groups (4-6 days after injection, CT: n=5, LPS: n=6). Values (mean±SEM) are expressed as a percentage of fascicles contained in each size group. Tukey-Kramer test. *p<0.05, **p<0.01. (P7: CT and LPS+Lf: n=5, LPS: n=10, LPS+Lf: n=11). (C) Representative immunoperoxidase labeling of SMI-312 in the striatum at P24. Bar = 200 µm.

Ginet V. et al, Biofactors, 2016
Inflammatory injury in the P3 rat + Lf

Ginet V. et al, Biofactors, 2016
Inflammatory injury in the P3 rat + Lf

Ginet V. et al, Biofactors, 2016

g-ratio: axon diameter/total fiber diameter
Inflammatory injury in the P3 rat + Lf

Ginet V. et al, Biofactors, 2016
## Inflammatory injury in the P3 rat + Lf

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Putative role</th>
<th>Reg.</th>
<th>LPS vs. Control</th>
<th>LPS-Lf vs. Control</th>
<th>LPS-Lf vs. LPS</th>
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<tbody>
<tr>
<td>Mac</td>
<td>Marker of tissue damage</td>
<td>Hp/St</td>
<td>↑</td>
<td>NC</td>
<td>↓</td>
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<tr>
<td>Ins</td>
<td>Glial marker/required for cell growth</td>
<td>St</td>
<td>↓</td>
<td>NC</td>
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<tr>
<td>PE</td>
<td>Component of cell membrane</td>
<td>St</td>
<td>↑</td>
<td>↑</td>
<td>NC</td>
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<tr>
<td>Total Cho</td>
<td>Components of cell membranes</td>
<td>Hp</td>
<td>↓</td>
<td>↓</td>
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<td></td>
<td></td>
<td>St</td>
<td></td>
<td></td>
<td>NC</td>
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<tr>
<td>GABA</td>
<td>Primary inhibitory neurotransmitter</td>
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<td></td>
<td></td>
<td>St</td>
<td></td>
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<tr>
<td>Glu</td>
<td>Excitatory neurotransmitter</td>
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<td>Glu+Gln</td>
<td>Involved in neurotransmission</td>
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<td>Glu-Gln cycle (neurons and glia)</td>
<td>Hp</td>
<td>NC</td>
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<td>NC</td>
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<tr>
<td>Total NAA</td>
<td>Neuronal damage/suffering</td>
<td>Hp</td>
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<td>NC</td>
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<tr>
<td></td>
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<td>St</td>
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<td>NC</td>
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<tr>
<td>Asc</td>
<td>Antioxydant</td>
<td>St</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>GSH</td>
<td>Antioxydant located in astrocytes</td>
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<td>↓</td>
<td>NC</td>
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<td>Lac</td>
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<td>Energetic status of the brain</td>
<td>Hp</td>
<td>↓</td>
<td>NC</td>
<td>↑</td>
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</table>

Ginet V. et al, Biofactors, 2016

TOTAL 17 7 9
Conclusion

Lactoferrin given through lactation showed neuroprotective effects after injury

- In IUGR rat pups
- In HI injury at P3
- In LPS inflammatory injury at P3

Anti-oxidative, anti-inflammatory properties and role in iron homeostasis of lactoferrin is likely to be involved in neuroprotection after HI injury in the developing brain

Promising approach ➜ Clinical trial?

Ochoa, T. and Sizonenko S.V., 2016, Biochemistry and Cell Biology
Clinical Trials in preterms infants

<table>
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<tr>
<th></th>
<th>Status</th>
<th>Title</th>
<th>Condition</th>
<th>Interventions</th>
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<td>1</td>
<td>Recruiting</td>
<td>Lactoferrin or Progesterone for Prevention of Preterm Delivery</td>
<td>Preterm Delivery</td>
<td>Drug: Lactoferrin; Drug: Progesterone</td>
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<td>2</td>
<td>Not yet recruiting</td>
<td>Oral Lactoferrin Supplementation for Prevention of Sepsis in Preterm Neonate</td>
<td>Neonatal Sepsis</td>
<td>Dietary Supplement: Lactoferrin; Dietary Supplement: Placebo</td>
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<td>3</td>
<td>Unknown †</td>
<td>Supplementation With Lactoferrin in Preterm Newborns</td>
<td>Prematurity; Low Birth Weight</td>
<td>Drug: Lactoferrin</td>
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<tr>
<td>4</td>
<td>Unknown †</td>
<td>Study of Talactoferrin Oral Solution for Nosocomial Infection in Preterm Infants</td>
<td>Nosocomial Infections</td>
<td>Drug: Talactoferrin; Drug: Placebo</td>
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<td>5</td>
<td>Completed</td>
<td>Pilot Study: Lactoferrin for Prevention of Neonatal Sepsis</td>
<td>Sepsis</td>
<td>Dietary Supplement: lactoferrin; Dietary Supplement: Maltodextrin</td>
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