Developing brain and systemic inflammation: a “Toll-like” link with consequences

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i sal Ivan Östholm, Medicinaregatan 13, fredagen den 16 juni 2017 kl. 13:00

av

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Avhandlingen baseras på följande arbeten:

I. Stridh L, **Mottahedin A**, Johansson ME, Valdez RC, Northington F, Wang X, Mallard C. *Toll-like receptor-3 activation increases the vulnerability of the neonatal brain to hypoxia-ischemia.* Journal of Neuroscience, 2013. 33(29): p. 12041-51.

II. **Mottahedin A**, Svedin P, Nair S, Mohn CJ, Wang X, Hagberg H, Ek J, Mallard C. *Systemic activation of Toll-like receptor 2 suppresses mitochondrial respiration and exacerbates hypoxic-ischemic injury in the developing brain.* Journal of Cerebral Blood Flow and Metabolism. 2017 Jan 1:271678X17691292.

III. **Mottahedin A**, Smith PL, Hagberg H., Ek CJ, Mallard C. *TLR2-mediated leukocyte trafficking to the developing brain.* Journal of Leukocyte Biology. 2017 Jan;101(1):297-305.

IV. **Mottahedin A**, Ek J, Truvé K, Hagberg H, Mallard C. *Differential analysis of TLR2- versus TLR4-induced alterations in transcriptome of choroid plexus reveals leukocyte trafficking mechanisms.* Manuscript.

V. **Mottahedin A**, Blondel S, Ek J, Babikian A, Hagberg H, Mallard C, Ghersi Egea JF, Strazielle N. *N-acetylcysteine inhibits TLR2-mediated neutrophil transmigration through the choroid plexus.* Manuscript.

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ABSTRACT

The developing brain is vulnerable to external insults, and perinatal brain injury (PBI) is a major cause of life-long neurological syndromes such as cerebral palsy. Currently, no pharmaceutical intervention is available. Hypoxia/ischemia (HI), infections and inflammation are implicated in the pathogenesis of PBI. However, the crosstalk between these etiologies is not fully understood. Toll-like receptors (TLR) 3 and TLR2 are responsible for sensing viral and bacterial infections and initiating the inflammatory response. The aim of this thesis was to investigate the effect of systemic inflammation induced by activation of these TLRs on neonatal HI injury. We demonstrate that intraperitoneal administration of TLR3 and TLR2 ligands (PolyI:C and P3C, respectively) prior to HI increase the brain injury in neonatal mice. PolyI:C and P3C induced neuroinflammation and altered microglial phenotype as assessed by RT-qPCR, multiplex cytokine assay or flow cytometry. PolyI:C also upregulated the pro-apoptotic gene, *Fasl,* expression and reduced activation of pro-survival signaling molecule Akt. On the other hand, P3C suppressed mitochondrial respiration, a major mechanism of cellular energy production. P3C, unlike other TLR agonists, induced marked infiltration of leukocytes to the cerebral spinal fluid and brain of neonatal mice and rats. Confocal microscopy, Cre recombinase-mediated gene targeting and *in vitro* cell transmigration assay revealed choroid plexus as a site of leukocyte entry. RNA sequencing of the choroid plexus and transcriptome cluster analysis and Ingenuity Pathway Analysis revealed potential mechanisms of leukocyte infiltration including a specific chemotaxis signature and cytoskeleton-related pathways. Finally, we show that N-acetylcysteine treatment inhibits TLR2-mediated leukocyte trafficking *in vivo* and *in vitro*.

To conclude, this thesis describe a TLR-mediated link between systemic inflammation and developing brain with detrimental consequences on HI brain injury suggesting potential novel therapeutic strategies.

Keywords: neonatal brain injury, hypoxia-ischemia, inflammation, infection, Toll-like receptor, choroid plexus

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