Göteborg, 2024

Perinatal brain damage – phagoptosis and neuroprotection

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, den 26 april 2024, klockan 09.00

av Andrea Jonsdotter

Fakultetsopponent:

Docent Magnus Gram, Institutionen för kliniska vetenskaper, Lunds Universitet, Lund, Sverige

Avhandlingen baseras på följande delarbeten

I. Rocha-Ferreira E, Poupon L, Zelco A, Leverin A-L, Nair S, Jonsdotter A, Carlsson Y, Thornton C, Hagberg H*, Rahim A*. Neuroprotective exendin-4 enhances hypothermia therapy in a model of hypoxic-ischaemic encephalopathy. Brain 2018 Oct 1;141(10):2925-2942. doi: 10.1093/brain/awy220 *equal contribution

II. Jonsdotter A, Rocha-Ferreira E, Hagberg H, Carlsson Y. Maternal and fetal serum concentrations of magnesium after administration of a 6-g bolus dose of magnesium sulfate ($MgSO_4$) to women with imminent preterm delivery. Acta obstet Gynecol Scand. 2022 Aug;101(8):856-861. doi: 10.1111/aogs.14372. epub 2022 May 2

III. Jonsdotter A, Hagberg H, Leverin A-L, Joakim Ek, Kerstin Ebefors, Rocha-Ferreira E, Carlsson Y. Mer-TK and the role of phagoptosis in neonatal hypoxia-ischemia. Manuscript

IV. Jonsdotter A, Carlsson Y, Leverin A-L, Svedin P, Eberfors K, Ek J, Hagberg H*, Rocha-Ferreira E*. Exendin-4 improves neurodevelopmental outcome in a preterm rat model of germinal matrix hemorrhage. *Manuscript*

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER

GÖTEBORGS UNIVERSITET

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Perinatal brain damage – phagoptosis and neuroprotection

Andrea Jonsdotter

Department of Obstetrics and Gynecology, Institute of Clinical Science, Sahlgrenska Academy, Gothenburg University, Sverige, 2024.

Abstract

Background: Neonatal encephalopathy is a serious outcome in term infants affecting 1-2/1000 live births and is often caused by perinatal hypoxia-ischemia (HI). However, brain injury is multifactorial and infection in the mother during pregnancy could cause or aggravate brain damage. Cerebral palsy (CP) is a known complication due to brain damage and occurs in both term and preterm infants. In preterm infants the risk of CP is inversely proportional to gestational age.

Aim: To better understand mechanisms of brain damage in preterm and term infants and thereby develop new strategies for neuroprotection.

Material and methods: Paper I, III and IV are animal experiments on mice and rats. **Paper I** focus on the neuroprotective effect of the glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 after term HI in neonatal mice, with or without therapeutic hypothermia. In **Paper IV** the neuroprotective effect of exendin-4 in a preterm model of cerebral GMH is addressed in rats. **Paper III** aims to evaluate the neuronal cell death in a mouse model of HI after gene deletion of the phagocytic receptor Mer-tyrokine kinase (Mer-TK). **Paper II** presents a clinical study where a bolus dose of magnesium sulfate ($MgSO_4$) is administered to pregnant women with imminent risk of preterm delivery to determine the concentration of serum magnesium (s-Mg) in both the mothers and the umbilical cords of the infants.

Results: Paper I: Exendin-4 treatment alone showed significant neuroprotection and it enhanced the cerebroprotective effect of hypothermia ($p < 0.0001$). Tissue infarction was significantly reduced after only one dose of exendin-4 injection (saline: 50% ± 6.9%) and (exendin-4: 17% ± 8.6%) ($p=0.03$) and adding the dose regime every 12 h over a 48h period reduced brain injury further to 2% ± 1.8% ($p=0.02$). **Paper IV:** This study shows that exendin-4 reduced brain injury after GMH. The neuroprotective effect was detected as early as 48 h after GMH ($p=0.05$ in striatum and $p=0.04$ in hippocampus) and was sustained until adulthood (P40, $p < 0.0001$). Exendin-4 improved motor skills significantly in different behavioral tests including rotarod (P20, $p=0.003$; P40, $p < 0.0001$), eye opening (P14, $p=0.05$) and negative geotaxis (P8, $p=0.05$). **Paper III:** Genes related to phagoptosis including MerTK and Gas-6 were upregulated at 6-72h after HI in the brain. Brain injury was reduced by 48% in gray matter ($p=0.002$) in MerTK knock-out (KO) vs wild-type (WT) animals and in white matter by 32% ($p=0.04$). Immunostaining of neurons and microglia indicated less neuronal phagocytosis by microglia in MerTK KO vs WT animals, ($p= 0.03$). **Paper II:** A bolus dose of 6 g of $MgSO_4$ seems to be well tolerated by the women and no extra surveillance is needed. The target concentration of s-Mg was reached in the blood of most of the women and the concentrations were low (0.87 to 1.4 mmol/l) in the umbilical cord at birth unlikely to adversely affect the newborn infants.

Conclusion: This thesis shows that: 1. the GLP-1 receptor agonist exendin-4 provides strong neuroprotection in rodent neonatal models of HI and GMH, and has, suggestedly, potential for clinical implementation; 2. gene deletion of the microglial MerTK receptor reduces both microglial phagocytosis of neurons and brain injury implicating involvement of phagoptosis in HI; 3. a 6 g bolus of $MgSO_4$ is well tolerated in pregnant women and is not likely to adversely affect the newborn preterm infant. This regimen is now established and implemented in all Swedish hospitals that provide care for deliveries up to 32 weeks of gestation.

Keywords: hypoxia-ischemia, germinal matrix hemorrhage, exendin-4, Mer-TK, magnesium sulfate, preterm, cerebral palsy, hypothermia, phagocytosis, microglia.

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