Inflammation in experimental neonatal brain injury and in a clinical study of preterm birth; Involvement of galectin-3 and free radical formation

Christina Doverhag

Perinatal Center, Department of Physiology, Institute of Neuroscience and Physiology,
The Sahlgrenska Academy, University of Gothenburg, Box 432, 405 30 Göteborg, Sweden

Abstract

Introduction; Intrauterine infection/inflammation is associated with preterm delivery (PTD) and severe neonatal morbidity. Inflammation is also important in the secondary neurotoxic cascade leading to perinatal brain injury. The aims were to investigate (a) the novel inflammatory marker galectin-3 that affects accumulation, apoptosis and activation of inflammatory cells and (b) free radical formation in particular by the enzyme NADPH-oxidase responsible for free radical formation in inflammatory cells, in threatening PTD (III) and in the development of experimental perinatal brain injury (I,II).

Materials and methods; Amniotic fluid (AF) and placentas were obtained from 83 women with threatening PTD presenting with preterm labour (PTL) or premature prelabour rupture of membranes (pPROM) and from 15 term controls. AF was analysed for galectin-3, ascorbyl radicals and antioxidative capacity (AOC) and results were correlated to signs of intrauterine infection/inflammation, PTD and severe neonatal morbidity (III). In the experimental studies, models of term hypoxia-ischemia (HI) (I,II) and of excitotoxic white matter injury (I) were used in knock-out (KO) mice for galectin-3 (II) and $gp91^{phox}$ (a subunit in NADPH-oxidase) (I) and together with two inhibitors of NADPH oxidase (I). Lesion size, inflammation (microglia accumulation, inflammatory mediators), free radical formation, apoptosis and trophic factors were studied. In vitro studies were used to investigate free radical formation in microglial cells (I) and the effect of AF on pre-activated neutrophils (III).

Results; Galectin-3, ascorbyl radicals and AOC were detected in all AF samples. Women with threatening PTD and infection/inflammation had higher levels of galectin-3 and slow AOC but lower levels of ascorbyl radicals than term controls but without correlation to PTD. Mothers of girls had higher levels galectin-3 than mothers of boys. AF also quenched free radical formation in primed neutrophils in vitro. Women with PTL did not differ from women with pPROM. Only AOC was increased in mothers to infants with severe morbidity (III). After experimental HI, mRNA expression of galectin-3 and NADPH-oxidase subunits (I,II) as well as galectin-3 protein expression were increased (II). Galectin-3 KO mice had reduced injury but increased microglial density and reduced expression of inflammatory factors (MMP-9) and markers for oxidative stress (nitrotyrosine). The protection was more pronounced in males. Apoptosis was altered in un-operated KO mice but not after HI. No difference was seen for trophic factors (II). Pharmacological inhibition of NADPH-oxidase reduced free radical formation in vitro and markers of oxidative stress in vivo, but resulted in an increase in apoptotic markers and lesion size after excitotoxic injury. Genetic inhibition did not reduce injury but resulted in an increase of the inflammatory markers galectin-3 and IL-1β (I).

Conclusion; Galectin-3 is increased in women with threatening PTD and associated infection/inflammation,but our findings suggest a strong antioxidative capacity and not an increased oxidative stress as previously suggested (III). Galectin-3 contributes to neonatal brain injury by modulating the inflammatory response rather than affecting apoptosis or trophic factors and the mechanisms may be sex dependent (II). Contrary to findings in the adult brain, perinatal brain injury was unaltered or aggravated after genetic and pharmacological NADPH-oxidase inhibition (I).

Key words: preterm labour, premature prelabour rupture of membranes, chorioamnionitis, hypoxia-ischemia, neonatal brain injury, NADPH oxidase, galectin-3, MMP-9, ascorbyl radicals, antioxidative capacity.

ISBN; 978-91-628-7864-1

Inflammation in experimental neonatal brain injury and in a clinical study of preterm birth;

Involvement of galectin-3 and free radical formation

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, torsdagen den 28.e januari 2010, kl 09.00

av

Christina Doverhag

Fakultetsopponent:
Professor Donald Peebles, MD, PhD
Department of Maternal and Fetal Medicine,
University College London, England

Avhandlingen bseras på följande delarbeten;

I. **Doverhag C**, Keller M, Karlsson A, Hedtjarn M, Nilsson U, Kapeller E, Sarkozy G, Klimaschewski L, Humpel C, Hagberg H, Simbruner G, Gressens P, Savman K. **Pharmacological and genetic inhibition of NADPH oxidase does not reduce brain damage in different models of perinatal brain injury in newborn mice**. Neurobiol Dis. 2008 Jul;31(1):133-44. Epub 2008 Apr 25.

II. **Doverhag C**, Poirier F, Hedtjarn M, Hagberg H, Karlsson A, Savman K. **Galectin-3 contributes to neonatal hypoxic-ischemic brain injury.** *(in press)*

III. **Doverhag C**, Jacobsson B, Holst RM, Wennerholm UB, Nilsson UA, Hagberg H, Karlsson A, Savman K. **Galectin-3 and oxidative stress in association with chorioamnionitis and preterm birth** *(manuscript)*

Göteborg 2010

UNIVERSITY OF GOTHENBURG