

# Galectin-3 in neonatal hypoxic-ischemic brain injury

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**Introduction:** Inflammation is important in the development of brain injury after neonatal hypoxia-ischemia (HI). Galectin-3 is a protein with newly discovered inflammatory features. It is a strong chemotactic factor for neutrophils (1) as well as for monocytes and macrophages (2), and has the ability to activate NADPH-oxidase in inflammatory cells resulting in a massive release of oxygen free radicals (3). Galectin-3 is expressed in microglial cells both *in vitro* and *in vivo* (4,5) but its role in brain injury has not been studied.

**Aim;** To investigate the expression of galectin-3 and its role in inflammatory activation and development of injury after neonatal HI.

**Materials and methods:** HI was induced in C57/Bl6 mice at P9 by ligation of the left common carotid artery followed by global hypoxia (10% oxygen) for 60 min. Gene expression in brain tissue was examined, by microarray (Affymetrix©) in injured compared with uninjured hemisphere at 2, 8, 24 and 72 h after HI. In addition, HI was induced at P9 in C57/Bl6 wild type (+/+) mice and their galectin-3 knock-out (-/-) littermates. Pups were sacrificed at P16 and the brains perfusion-fixed and cut into 5µM coronal sections for evaluation of injury. Pups were also sacrificed 3, 8, 24 and 96 h after HI for evaluation of galectin-3 and inflammatory activation. For localization of galectin-3, double immunolabeling was performed with iba-1 and lectin (microglia), GFAP (astrocytes) as well as NeuN (neurons). For evaluation of injury approx 10 sections evenly distributed throughout the brain were stained for MAP-2 and positive areas were measured using MicroImage©. The area of the infarction was expressed as percentage tissue loss in the left injured hemisphere compared with the uninjured right hemisphere. Infarction area in (+/+) and (-/-) mice were compared. White matter injury was assessed at 24, 96 and 7days after injury by MBP-staining and tissue loss calculated as above. Inflammation was assessed by the number of iba-1 positive cells at 24h after HI and by measuring iba-1 positive areas at 96 h and 7 d after HI.

**Results:** The gene expression of galectin-3 was increased 4, 9 and 12-fold at 8, 24 and 72 h after HI in the injured hemisphere. There were no or very few galectin-3 positive cells 3h and 8h after HI. The number of galectin-3 positive cells increased significantly at 24h and reached a peak with massive expression after 96 h that persisted up to 7 days after HI. Iba-1 and lectin positive cells stained for galectin-3. The overall infarction area was significantly smaller ( $p=0.03$ ) in mice lacking functional galectin-3. The reduction in infarction size was significant at brain levels encompassing hippocampus as well as striatum. At the more anterior and posterior levels no difference was seen between the groups. No difference was seen for MBP staining. Iba-1 positive area was increased in (-/-) mice compared to (+/+) mice at 7days after HI.

**Conclusion** Galectin-3 gene expression was upregulated after HI in the immature brain. Galectin-3 protein was found in microglial cells only and there was an intense staining from 24 h after HI and onward. Mice lacking galectin-3 were protected from HI injury in the important areas of striatum and hippocampus. Interestingly, the reduction in infarction was accompanied by an increased microglial density suggesting that galectin-3 might be involved in the inflammatory response as well as development of injury after neonatal HI.

**References:** 1.Colnot et al 1998 J Immunology 94(3): 290-296; 2.Sano et al. 2000 J Immunology 15; 165 (4):2156-2164; 3.Karlsson et al 1998 Blood 1; 91(9):3430-3438; 4.Walter et al. 2000 J Neurosci Res 15;61(4):430-435; 5.Pesheva et al. 1998. J Neurosci Res. 1;51(1):49-57