Apoptotic mechanisms in the neonatal brain following hypoxia-ischemia

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Introduction: Neonatal encephalopathy is often perinatally acquired and caused by hypoxia-ischemia (HI). Brain injury develops with a delay, over 12-48 hours, after the insult. Hypothermia, an established neuroprotective treatment, saves 1 infant in 9 from neurological deficits suggesting that there is room for further improvement. HI leads to cell death through multiple pathways, including apoptosis. The aim of this thesis was to investigate different apoptotic pathways and to explore possible apoptotic targets for future pharmacological treatment after perinatal brain injury. We investigated (I) the involvement of caspase-2 alone, (II) and in combination with hypothermia, (III) the role of c-Jun N-terminal kinase (JNK), and (IV) Cyclophilin D (CypD), a regulator of the mitochondrial membrane permeability transition pore.

Materials and methods: Wild type (WT) C57BL/6 and transgenic mice with gene deletion of caspase-2 (I, II) and CypD (IV) were used in the ibotenate (excitotoxic)-model (I), and/or Rice-Vannucci´s HI-model (I-IV) at postnatal day 5 (I) or 9 (I-IV). The mixed lineage kinase inhibitor CEP-1347 was used to explore the role of JNK after neonatal HI (III).

Results: Caspase-2-deficient mice demonstrated less gray and white matter injury after both neonatal HI and an excitotoxic insult (I). Hypothermia provided additional protection in caspase-2 deficient mice (II). CEP-1347 was neuroprotective in the immature brain, by reducing apoptosis and attenuating microgliosis (III). CypD gene deficiency enhanced HI injury and Bax inhibitory peptide (BIP) reduced injury in the immature brain, whereas CypD deletion protected and BIP had no effect on brain damage in the mature mouse brain. Apoptosis was more pronounced in the immature CypD deficient mice than in WT controls, while adults showed minimal apoptotic activation.

Conclusion: Apoptosis has a more prominent role in the immature brain and different pathways leading to cell death after HI are at play in the immature as compared to the adult brain. This suggests that different pharmacological interventions are required in the immature and the mature brain. We suggest that caspase-2 as well as Bax dependent mitochondrial permeabilization are important neuroprotective targets in neonatal HI brain injury.

Key words: hypoxia-ischemia, brain, caspase-2, cyclophilin D, MLK, neonatal
ISBN; 978-91-628-8334-8

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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, fredagen den 4 november 2011, kl 09.00

av

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The thesis is based on the following papers:

I. **Carlsson Y**, Schwendimann L, Vontell R, Rousset CI, Wang X, Lebon S, Charriaut-Marlangue C, Supramaniam V, Hagberg H, Gressens P, Jacotot E. **Genetic Inhibition of Caspase-2 Reduces Hypoxic-Ischemic and Excitotoxic Neonatal Brain Injury.** Ann Neurol. 2011 Mar 28. doi: 10.1002/ana.22431

II. **Carlsson Y**, Wang X, Schwendimann L, Rousset CI, Jacotot E, Gressens P, Thoresen M, Mallard C, Hagberg H. **Combined effect of hypothermia and caspase-2 gene deficiency on neonatal hypoxic-ischemic brain injury.** Submitted

III. **Carlsson Y**, Leverin AL, Hedtjärn M, Wang X, Mallard C, Hagberg H. **Role of mixed lineage kinase inhibition in neonatal hypoxia-ischemia.** Dev Neurosci. 2009;31(5):420-6

IV. Wang X, **Carlsson Y**, Basso E, Zhu C, Rousset CI, Rasola A, Johansson BR, Blomgren K, Mallard C, Bernardi P, Forte MA, Hagberg H. **Developmental shift of cyclophilin D contribution to hypoxic-ischemic brain injury.** J Neurosci. 2009;29(8):2588-96

Göteborg 2011

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