

"Heijnen, C." <C.Heijnen@umcutrecht.nl>

## **A dual role of the NF-kappaB pathway in neonatal hypoxic-ischemic brain damage.**

Annemieke Kavelaars<sup>1</sup>, Cora H. Nijboer<sup>1,2</sup>, Floris Groenendaal<sup>2</sup>, Michael J. May<sup>3</sup>, Frank van Bel<sup>2</sup>, Cobi J. Heijnen<sup>1</sup>

<sup>1</sup>Laboratory of Psychoneuroimmunology and <sup>2</sup>Department of Neonatology, University Medical Center Utrecht, Lundlaan 6, 3584 EA Utrecht, The Netherlands, <sup>3</sup>Department of Animal Biology, University of Pennsylvania, School of Veterinary Medicine, 3800 Spruce Street, Philadelphia, PA 19104, USA.

**Background and Purpose:** NF- $\kappa$ B is a transcription factor that regulates inflammatory and apoptotic pathways. We described earlier that, in a model of neonatal hypoxic-ischemic (HI) brain damage, intraperitoneal administration of the NF- $\kappa$ B inhibitor TAT-NBD at 0 and 3h after the insult had a strong neuroprotective effect. Here we further delineated the pathophysiologic role of NF- $\kappa$ B in neonatal HI cerebral damage.

**Methods:** Brain damage was induced in P7 rats by unilateral carotid artery occlusion and hypoxia. We determined cerebral damage, NF- $\kappa$ B activity, cytokine mRNA, and pro- and anti- apoptotic molecules. In vitro effects of TAT-NBD were determined using primary neurons and cell lines.

**Results:** HI induced two peaks of cerebral NF- $\kappa$ B activity at 3-6 and 24h post-HI. Neuroprotective 0/3h TAT-NBD treatment only inhibited early NF- $\kappa$ B. However, inhibition of both early and late NF- $\kappa$ B-activity by 0/6/12h TAT-NBD or only late NF- $\kappa$ B activity by 18/21h TAT-NBD aggravated damage. 0/6/12 h TAT-NBD did not prevent HI-induced upregulation of cytokines at 24h after HI. Protective 0/3h TAT-NBD treatment prevented nuclear accumulation of p53 at 24h after HI. Nuclear p53 was not reduced after 0/6/12h TAT-NBD. Prolonged TAT-NBD increased the pro-apoptotic factor PUMA and reduced the anti-apoptotic factors Bcl-2 and Bcl-xL. Also in neuronal cultures prolonged TAT-NBD exposure overruled protective short-term TAT-NBD treatment.

**Conclusions:** Early NF- $\kappa$ B activation is responsible for brain damage. Late NF- $\kappa$ B provides endogenous neuroprotection by upregulating anti-apoptotic molecules. Inhibition of early NF- $\kappa$ B activity is neuroprotective only when late NF- $\kappa$ B activity is maintained. Moreover, cerebral cytokine production can occur independently of NF- $\kappa$ B.