306 A NOVEL ANTI-OXIDANT TO COUNTERACT OXIDATIVE STRESS DURING RESUSCITATION AFTER BIRTH ASPHYXIA

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Background and Aims Oxidative stress contributes to tissue damage after perinatal asphyxia. The thiol-containing free radical scavenger *N*-acetylcysteine amide (NACA) is a promising new anti-oxidant with good penetration into the mitochondria. The objective was to investigate the protective effect of NACA in a piglet model of birth asphyxia.

Methods Anesthetized newborn piglets (n=51) were subjected to global hypoxemia and block-randomized to either

1. intravenous administration of NACA 300 mg/kg and resuscitated with 21% oxygen for 30 min,
2. saline and 21% oxygen,
3. NACA and 100% oxygen or
4. saline and 100% oxygen.

After resuscitation, the piglets were followed for 9 hours and samples for several markers of injury and oxidative stress were collected. Reported here are clinical parameters and measurements of reduced to oxidized glutathione (GSH/GSSG).

Results Thirty minutes after end-resuscitation metabolic acidosis was less pronounced in the 100%-NACA group compared to 100%-oxygen-alone (lactate 8.1 ± 2.6 vs 10.9 ± 3.4 , $p < 0.05$). This difference was not shown for the 21%-oxygen groups. Mean arterial blood pressure and hemoglobin levels remained similar between the groups. The GSSG values were generally low. At end-resuscitation GSH was lower in 100%-NACA compared to 100%-oxygen-alone group (164 ± 111 vs 255 ± 113 μmol , $p < 0.05$) and delta-GSH during resuscitation greater (143 ± 49 vs 32 ± 66 μmol $p < 0.001$).

Conclusions The data indicate that NACA may enhance immediate recovery, improve mitochondrial glutathione metabolism and restore the cell to a normal metabolism following asphyxia and resuscitation. Upcoming analyses of histopathology and injury markers will further elucidate neuroprotective effect of NACA treatment following birth asphyxia.

307 EFFECTS OF THE ISCHEMIC POSTCONDITIONING IN A NEONATAL STROKE MODEL

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The lack of efficient neuroprotective strategies for neonatal stroke could be ascribed to pathogenic ischemic processes differentiating adults and neonates. In the present study, we tested the hypothesis that ischemic postconditioning applied immediately after ischemia provides neuroprotection in a neonatal stroke model. Neonatal ischemia was generated by permanent occlusion of the left distal middle cerebral artery combined with 50 min of occlusion of both common carotid arteries (CCA) in P7 rats. Postconditioning was performed by repetitive brief release and occlusion (30 s, 1 or 5 min) of CCA after 50 min of CCA occlusion. Alternative reperfusion was generated by controlled release of the bilateral CCA occlusion. Blood-flow velocities in the left internal carotid artery were measured using ultrasound imaging with sequential Doppler recordings. Cortical

perfusion was measured using laser Doppler. Local cerebral blood-flow was measured by iodo[¹⁴C]antipyrine autoradiography. None of the procedures of serial mechanical interruptions of blood flow applied at reperfusion induced a reduction of infarct volume after 72 hours. In contrast to adult ischemia, a gradual perfusion was found during early re-flow both in the left internal carotid artery and in the cortical penumbra. No hyperemia was detected on autoradiograms. In addition, vasodilation to hypercapnia remained unchanged. Absence of acute hyperemia during early CCA re-flows and absence of increased cerebrovascular reactivity could at least in part explain the inefficiency of ischemic postconditioning in the developing brain.

308 TLR3 ACTIVATION INCREASES THE VULNERABILITY OF THE NEONATAL BRAIN TO HYPOXIA-ISCHEMIA

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Background Activation of the innate immune system by bacterial products, via toll-like receptors (TLRs), has been shown to play a role in neonatal hypoxic-ischemic (HI) brain injury in mice. Less is known about how viral products affects neonatal HI. The aim of this study was to investigate the role of a synthetic mimic of double stranded RNA viral products (Poly I:C) in neonatal HI brain injury and whether these effects were dependent on TLR3 via its specific adaptor protein TRIF.

Method Neonatal wildtype (WT) and TRIF knock out (KO) mice were injected with Poly I:C and 14h later subjected to unilateral HI (10% O₂, 36°C, 50 min). To evaluate brain damage, immunostaining for myelin basic protein (MBP) and microtubule associated protein-2 (MAP-2) were quantitatively analyzed 5 days after Poly I:C/HI. Cerebral mRNA expression was investigated for IFN- β , IL-6, IL-1 β , TNF- α , MCP-1, IP-10 and Fas with RT-qPCR.

Results Poly I:C pre-treatment increased HI brain injury in WT mice, which was blocked in TRIF KO mice. Gene expression analyses showed a TRIF specific upregulation of IFN- β , IL-6, IL-1 β , TNF- α , chemokines IP-10 and MCP-1 and the apoptotic mediator Fas.

In summary, the study shows that activation of TLR3 prior to HI increases neonatal brain injury. The sensitizing effect of Poly I:C was associated with release of a range of type I interferons, pro-inflammatory cytokines and chemokines. The results may indicate that viral infections in the neonate could have great impact on HI brain injury in the newborn.

309 TUMOR NECROSIS FACTOR-INDUCIBLE GENE 6 PROTEIN: A NOVEL NEUROPROTECTIVE FACTOR AGAINST INFLAMMATORY DEVELOPMENTAL BRAIN INJURY

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Background and Aims An important factor of developmental brain injury is inflammation. It has been shown that tumor necrosis factor-inducible gene 6 protein (TSG-6) has anti-inflammatory effects in several inflammatory conditions. Nothing is known so far about the role of TSG-6 in the developing brain, its impact on inflammation and its therapeutic potential.

Methods PCR, Western Blotting and Immunohistochemistry was performed according to standard protocols. Brain hemispheres of untreated Wistar rats (p1-p15) were evaluated under developmental aspects of TSG-6. LPS-treated rats (0.25mg/kg LPS i.p. on p3) were evaluated under pathological aspects of TSG-6. To evaluate whether exogenous rhTSG-6 reduces inflammatory-induced brain injury,

newborn Wistar rats, exposed to LPS at p3, were treated with rhTSG-6 i.p. (four repetitive doses of 2.25mg/kg every 12h, first dose three hours before LPS-injection).

Results Investigations of TSG-6's developmental brain expression showed a linear increase from p1 to p15 on gene level. Additionally, different expression was detected in Cortex, Thalamus and Striatum on gene level at p6. Expression of TSG-6 after LPS treatment (0–24h) was significantly increased on gene level and tendentially on protein level. cCaspase-3, a marker of apoptosis, showed a significant down-regulation of >30% under additional TSG-6 treatment versus sole LPS exposure (n=12–14, p=0,025).

Conclusions TSG-6 Expression is developmentally regulated and increased after LPS exposure. The reduction of activated Caspase-3 demonstrates the neuroprotective potential of exogenous TSG-6 administration in inflammatory-induced developmental brain injury.

310 METHEMOGLOBIN DETERMINES DEGREE OF PRO-INFLAMMATION AND CHOROID PLEXUS APOPTOSIS FOLLOWING INTRAVENTRICULAR HEMORRHAGE IN PRETERM RABBIT PUPS

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Background and Aims Inflammation following intraventricular hemorrhage (IVH) has been shown to cause periventricular brain damage. Released free hemoglobin may be an early up-stream initiator of inflammation in the intraventricular space and cause damage to the choroid plexus. We aimed to evaluate release of free hemoglobin, the relationship to inflammation in the intraventricular space and effects on choroid plexus epithelium following IVH.

Methods IVH was induced in preterm rabbit pups. Intraventricular samples of cerebrospinal fluid (CSF) were obtained under guidance of high-frequency ultrasound at 24, 48 and 72h following IVH for measurement of free oxyhemoglobin (OxyHb), methemoglobin (MetHb) and TNF α . Analysis of gene expression (RT-PCR) for TNF α , IL-1b and heme-oxygenase-1 (HO-1) and immunostaining with electronmicroscopy (EM) was performed in choroid plexus obtained at 72h.

Results Intraventricular CSF concentrations of free OxyHb remained stable whereas those of MetHb and TNF α increased from 24 to 72 h. There was a highly positive correlation between concentrations of MetHb and TNF α in CSF at 72h (r= 0.98, p<0.0001). Levels of mRNA for HO-1, IL-1 b and TNF- α were up-regulated in choroid plexus following IVH as compared to controls (all p<0.01). EM showed choroid plexus epithelium with fragmented microvilli, apoptotic bodies, swollen mitochondria and increased epithelial expression of TNF α following IVH (fig. 1).

Abstract 310 Figure 1

Conclusions Amount of released free Hb, subsequently auto-oxidised to MetHb determines degree of pro-inflammation in intraventricular CSF following IVH. This is associated with severe apoptosis in choroid plexus epithelium.

311 THE SIGMA-RECEPTOR-AGONIST 4-PHENYL-1-(4-PHENYLBUTYL) PIPERIDINE (PPBP) DECREASES NEONATAL EXCITOTOXIC BRAIN INJURY AND DIMINISHES MICROGLIAL CELL ACTIVATION

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The pathogenesis of perinatal brain injury is multifactorial. In animal models of adult brain injury the sigma-1-receptor agonist 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) was shown to be neuroprotective due to modulation of inflammatory and apoptotic pathways and affection of excitotoxic signaling cascades.

In the current study we evaluated the effect of PPBP in a neonatal animal model of excitotoxic brain injury.

We performed an intracranial injection of ibotenate in CD1 mice on postnatal day 5 (P5) to induce excitotoxic injury. A single injection of vehicle or PPBP, in a dosage of 1, 5 or 50 μ g/g body weight, was applied intraperitoneally one hour after the insult. Primary study endpoints were evaluated 24 (P6) and 120 (P10) hours after the insult by analysis of lesion size, number of cells stained positively for cleaved caspase-3 and isolectin B4.

Systemic application of PPBP resulted in a significant reduction of lesion size in cortical gray matter in all dosages (p<0.05). and underlying white matter in 1 and 50 μ g/g (p<0.05) at P10. At P6 microglial cell activation was significantly diminished by PPBP in cortical gray (p<0,001) and underlying white matter (p<0.05). Lesion size on P6 and number of cells stained positively for activated caspase-3 at P6 was not affected by the treatment (p>0.05).

In this neonatal animal model we were able to demonstrate a beneficial effect of the sigma-1 receptor agonist PPBP. This data suggest that PPBP may be a potential therapeutic approach in neonatal brain injury.

312 INTERMITTENT HYPOXIA: EFFECTS ON BRAIN STEM OF OXIDATIVE STRESS AND NRF2 TRANSCRIPTION FACTOR ACTIVATION IN A RAT PUP MODEL

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Background Apnea of prematurity is a common condition in the neonatal period caused by immature brainstem respiratory neural output. Apneic episodes and resultant intermittent hypoxia are a potential cause of oxidative stress during this vulnerable developmental period.

Objective We tested the hypothesis that chronic intermittent hypoxia alters oxidative metabolism and resultant redox status in the medulla of rat pups.

Design/methods Litters of 10 rat pups and their dams were assigned to one of two groups: normoxia (controls) and intermittent hypoxia (Hx). Exposure occurred from postnatal day 1 for 7 days. Chronic intermittent hypoxia consisted of exposing rat pups and their dams to alternating cycles of nitrogen (N $_2$) and room air (RA): 45 seconds of hypoxia (nadir of 5% O $_2$) was administered every 5 minutes for 8 hours/day. For controls, animals were kept at RA. On the eighth day, brainstems were harvested and quickly snap frozen in liquid nitrogen. Reduced (GSH) and oxidized (GSSG) glutathione, and the GSH precursors -glutamyl-cysteine (-G-cysteine)