

50% of hypothermic piglets *c.f.* 83% in normothermic piglets. At 48h post insult both groups showed a maximum of epileptic activity. The neurobehavioral score in the normothermic piglets showed an earlier return to baseline compared to the hypothermic piglets.

Conclusions Electrographic seizure burden was decreased following TH. aEEG background pattern and neurobehaviour score recovered earlier in normothermic piglets, suggesting that in the clinical situation conclusions based on aEEG and neurological examination should not be performed too early.

303 HIGH DOSE INTRAVENOUS MELATONIN FOR AUGMENTATION OF HYPOTHERMIC NEUROPROTECTION LEADS TO HYPOTENSION

doi:10.1136/archdischild-2012-302724.0303

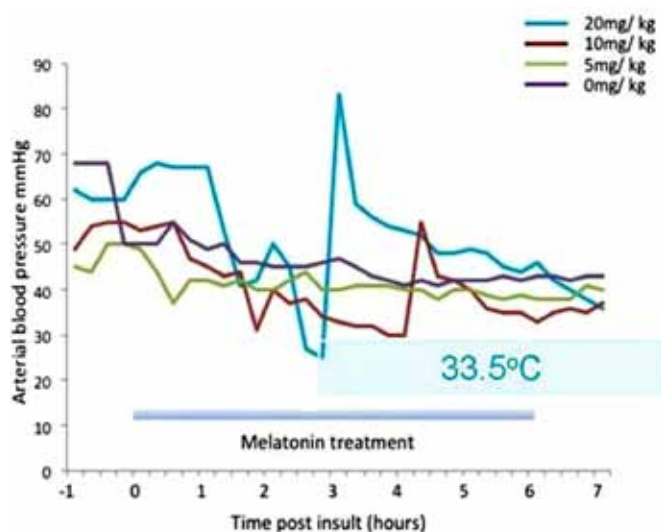
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Background Therapeutic hypothermia provides neuroprotection in infants with moderate to severe neonatal encephalopathy, however further treatments are necessary as hypothermic neuroprotection is not absolute.

Aim To assess the effect on mean arterial blood pressure of high dose intravenous melatonin (20, 10, 5 mg/kg) administered 10 minutes after hypoxia-ischemia.

Methods Male piglets underwent a hypoxic-ischemic insult and were then commenced on different doses of intravenous melatonin. Physiological measures such as mean arterial blood pressure and heart rate were measured. Hypothermia (core temperature 33.5°C) was induced at 2–26h after hypoxia-ischemia.

Results 20mg/kg melatonin induced a rapid reduction in blood pressure beginning at 1 hour following its administration (1–3h post administration; mean arterial blood pressure was reduced from 60 to 24mm Hg). 10mg/kg induced a gradual reduction in blood pressure at 1 hour post administration (1–4h post administration; mean arterial blood pressure was reduced from 55 to 31mm Hg). However treatment with either 0 or 5 mg/kg melatonin had no effect on mean arterial blood pressure.



Abstract 303 Figure 1 Effect of different Melatonin doses on mean BP

Conclusion Intravenous doses of melatonin (>5mg/kg) lead to hypotension following a hypoxic-ischemic insult when combined with hypothermia. Future pre-clinical studies of augmented hypothermic neuroprotection should be conducted using melatonin doses of 5mg/kg or less.

304 MINOCYCLINE ATTENUATES INJURY OF OLIGODENDROGLIAL PRECURSOR CELLS CAUSED BY OXYGEN-GLUCOSE-DEPRIVATION

doi:10.1136/archdischild-2012-302724.0304

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Background Oxygen-glucose deprivation (OGD) is a widely used *in vitro* model for ischemic brain injury, which leads to cell death. Prevention and attenuation of brain injury by the tetracycline-antibiotic minocycline has been largely attributed to suppression of microglial activation.

Methods Using mono-cultures of rat oligodendroglial precursor cells (OPC) exposed to oxygen-glucose deprivation (OGD), we investigated direct effects of minocycline on survival, proliferation, and maturation of oligodendroglial lineage cells.

Results OGD for 2 h decreased the number of A2B5+ cells and the amount of proliferating Ki67+ A2B5+ cells by 50% which was both attenuated by minocycline in a dose-dependent fashion. The reduced numbers of O4+ cells at 72 h and of O1+ cells at 120 h after OGD were partially restored by minocycline. In OPC, OGD caused an increase of reactive oxygen species (ROS) production and of TUNEL-positive cell numbers which was abolished by minocycline, possibly via induction of superoxide dismutase. Minocycline also prevented OGD-induced downregulation of the expression of the transcription factors Sox10 and Olig2, and of the maturation markers 2'3' cyclic nucleotide phosphodiesterase (CNP) and myelin basic protein (MBP).

Conclusion The results demonstrate that minocycline exerts direct protective actions on oligodendroglial lineage cells.

305 DOPAMINE REDUCES WHITE MATTER INJURY IN HYPOXIC-ISCHAEMIA IN THE PRETERM LAMB

doi:10.1136/archdischild-2012-302724.0305

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Background Dopamine is frequently used as inotropic agent in preterm infants. Its cardiovascular actions, as well as effects on neurovascular interactions may be neuroprotective during hypoxic-ischaemic events. Using a preterm lamb model we aimed to test the impact of intravenous dopamine on hypoxic-ischaemic brain injury.

Method Nine fetal lambs (91–93d gestation) were instrumented with catheters in carotid artery and jugular vein, and an umbilical cord occluder. Four days after surgery, intravenous dopamine (DA, 10 µg/kg/min, n=5) (or saline, n=4) was commenced. then a hypoxic-ischaemic insult was induced with umbilical cord occlusion for 25 mins. Infusions were continued for another 72 hours before euthanasia. Fetal brains were collected for immunohistopathology.

Results Dopamine infusion increased fetal heart rate (184±1 to 203±1 bpm, p<0.05) while arterial pressure was unchanged. Three animals in the DA group showed tachycardic response to cord occlusion, while the other two animals showed bradycardic response similar to the saline group. In the periventricular white matter, the saline group had higher number of microglia (lectin positive) than the DA group (10±3 vs 6±2 per 0.04mm², p<0.05). The saline group tended to have shorter myelinated fibre lengths (CNPase) compared with the DA group (15.0±2.0 vs 18.4±5.7µm respectively, p=ns). No histological differences were evident between DA animals exhibiting a tachycardic or bradycardic response during cord occlusion.

Conclusions Intravenous dopamine reduces hypoxic-ischaemic white matter injury in preterm lambs, independent of the cardiovascular response during the hypoxic-ischaemia.

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

doi:10.1136/archdischild-2012-302724.0306

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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

doi:10.1136/archdischild-2012-302724.0307

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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]antipyrene 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

doi:10.1136/archdischild-2012-302724.0308

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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

doi:10.1136/archdischild-2012-302724.0309

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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,