**ID:** 122  
**TITLE:** AMBULANCE EQUIVALENT VIBRATION INDUCED BRAIN INJURY IN THE DEVELOPING BRAIN: A TWO HIT MECHANISM?  
**AUTHORS:** Wan Teng Lee 1; Lara Shipley 1; Ian Bloor 1; Don Sharkey 1  
**AFFILIATIONS:** 1 Academic Child Health, School of Medicine, University of Nottingham, Nottingham, UK

**CONTENT:**

Inter-hospital transfer of preterm infants is associated with worse neurological outcomes. Chronic whole-body vibration (WBV) causes neurovascular injury, but it is unknown what impact a single, acute WBV exposure has on the developing brain as a potential mechanism of neuronal injury. Using our new rodent model of a single acute WBV exposure, as experienced during neonatal ambulance transfer, we have previously demonstrated outer cortical neuronal injury. We now hypothesise that the preterm phase of neuronal development is more susceptible to WBV induced traumatic brain injury (TBI) and aimed to explore neuronal injury in the whole developing brain.

Female Sprague-Dawley rats, at neurodevelopmental stages equivalent to the 32w preterm (postnatal day 7) or post-term (postnatal day 21) infant, were randomised into control (C) and WBV (V) groups. V groups were vibrated at 2m/s² for 90 minutes. 24 hours post-exposure, whole brain tissues were sectioned and TUNEL stained for apoptotic cells. Blinded histological quantification of apoptotic cells was performed within 4 cortical and 4 subcortical regions of interest and analysed using Mann-Whitney U test with significance set at P<0.05. This study was conducted in accordance to the Animal Act 1986.

Day 7 V pups had significantly more apoptotic cells in the outer cortex, mid-outer cortex, and corpus callosum/hippocampus regions compared to the C group (Table 1). Amongst day 21 pups, only the outer cortex of the V group showed significantly more apoptosis. In both V groups, apoptosis was maximised at the outer cortex and decreases toward deeper regions. Overall, more brain regions were significantly affected and at greater extent in day 7 V group than in day 21.

Ambulance equivalent WBV induces microscopic TBI within the developing brain, appearing particularly susceptible in the outer cortical layers. Deeper regional injuries in the corpus callosum and hippocampus could be related to poor stress response and subsequent poor cerebral autoregulation. These microscopic injuries could impact the long-term neurodevelopment of transported neonates even in the absence of intraventricular haemorrhage.

**IMAGES:**

https://www.eiseverywhere.com/eeselectv3/v3/events/351149/submission/files/download?fileID=61335e52c518cd7ed6209f172c0af93a-MjAxOS0wNSM1Y2UyNjY2YmQ1OWIy

Table 1: Percentage TUNEL +ve cells in day 7 and day 21 C and V groups.  
Data expressed as median (IQR). Statistical significance: ***p<0.001 C vs V, **p<0.01 C vs V, *p<0.05 C vs V

**COI:** None declared

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ID: 331

TITLE: RNA-BINDING PROTEIN RBM3 PREVENTS NEURAL STEM/PROGENITOR CELL (NSPC) APOPTOSIS AND PROMOTES NEURONAL DIFFERENTIATION AFTER HYPOXIC-ISCHEMIC (HI) BRAIN INJURY

AUTHORS: Xinzhou Zhu 1; Jingyi Yan 1; Catherine Bregere 2; Josef Kapfhammer 2; Raphael Guzman 2; Sven Wellmann 1,3

AFFILIATIONS: 1 University Children's Hospital of Basel (UKBB), Basel, Switzerland
2 Department of Biomedicine, University of Basel, Basel, Switzerland
3. University Children's Hospital of East Bavaria (KUNO), Regensburg, Germany

CONTENT:

Hypoxic-ischemic (HI) brain injury is one of the leading threats across all age groups from newborn infants to adults. Neural stem/progenitor cells (NSPCs) play critical roles in neuroregeneration after HI injury. Previously, the RNA-binding protein RBM3 has been shown to express in nestin-positive NSPCs and doublecortin (Dcx)-positive neuroblasts in rodent brain, but the functions of RBM3 in NSPCs remain largely unknown. In this study, we demonstrate that RBM3 can protect NSPCs against apoptosis and promote neurogenesis after HI injury.

RBM3 WT and KO mice were used in this study. For in vitro experiments, NSPCs were isolated from neurogenic niches. Cultured NSPCs were challenged with oxygen glucose deprivation (OGD) to mimic hypoxic ischemia. NSPC apoptosis was measured by TUNEL assay. Neuronal differentiation was performed after OGD by withdrawing growth factors. Immunostaining was used to assess neuronal differentiation. An in vivo HI model was applied by ligating the right common carotid artery of adult mice and exposing mice to 8% hypoxia for 20 min. The mice were recovered for 7 days with BrdU injection. The infarction volume was measured by Nissl staining. The numbers of TUNEL+ apoptotic NSPCs were counted. Newborn neuroblasts and neurons were stained with BrdU/Dcx or BrdU/NeuN antibodies, respectively.

The effects of RBM3 were detected in the two neurogenic niches, subventricular zone (SVZ) and dentate gyrus (DG).

1. In vitro, more apoptotic cells were observed in RBM3 KO NSPCs after OGD stress. Both SVZ- and DG-derived NSPCs showed similar results.
2. In vivo, RBM3 KO mice showed a larger infarction volume than WT mice after HI injury.
3. In vivo, apoptotic cells were in significantly higher numbers in the SVZ and DG regions of RBM3 KO mice after HI injury.
4. In vitro, less neuronal differentiation was observed from RBM3 KO NSPCs after OGD stress. Both SVZ- and DG- derived NSPCs showed similar results.
5. In vivo, the newborn neuroblasts (BrdU+Dcx+) and neurons (BrdU+NeuN+) were significantly fewer in the SVZ and DG regions of RBM3 KO mice after HI injury.

Our results show that the absence of RBM3 dramatically exacerbated NSPC apoptosis and inhibited the neuronal differentiation potential of NSPC after OGD stress in vitro. In an in vivo HI model, the insult caused larger infarction and more neuronal loss in RBM3-depletion mice. Neurogenesis was also impaired when RBM3 was depleted. Taken together, our data suggest that RBM3 protects NSPCs against apoptosis and promotes neurogenesis after HI injury.

COI: None declared
ID: 340

TITLE: CHANGES IN THE APPLICATION OF THERAPEUTIC HYPOTHERMIA IN NEWBORNS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY: A RETROSPECTIVE ANALYSIS

AUTHORS: Corline Parmentier 1; Linda de Vries 2; Mona Toet 3; Lauren Weeke 4; Floris Groenendaal 5

AFFILIATIONS: Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

CONTENT:

Several trials have shown that therapeutic hypothermia (TH) reduces death and disability in infants with moderate to severe hypoxic-ischemic encephalopathy (HIE). Increasing clinical experience with the application of TH and positive results concerning safety may have led to an increase in the use of TH in infants with milder HIE. The aim of this study was to determine whether the infants treated with TH during the initial years after the implementation of this neuroprotective intervention in our hospital were more severely affected by HIE than the infants cooled during the subsequent years.

We performed a retrospective, single center study in newborns with HIE who were treated with TH from February 2008 up to and including July 2017. Thompson scores, Sarnat scores and aEEGs of the infants treated during the first 57 months after the implementation of hypothermia in our hospital (February 2008 until October 2012) were compared to those from the infants treated during the subsequent 57 months (November 2012 until July 2017).

From February 2008 through July 2017, 222 newborns with HIE were treated with TH. Sarnat scores were documented for 215 newborns: 37 (17.2%) had mild HIE, 125 (58.1%) had moderate HIE and 53 (24.7%) had severe HIE. One hundred and seventeen infants were cooled in the first period, and 105 were cooled in the second period. The infants cooled in the second period had lower Thompson scores (median = 9, interquartile range 7 to 12) than the infants treated in the first period (median = 10, interquartile range 8 to 12), p = 0.002. The proportion of infants with mild HIE was lower in the first period (6.8%) than in the second period (29.6%), p < 0.001. There was no significant difference in the aEEG background patterns between the two periods (p = 0.571).

A substantial number of infants cooled in our hospital had mild HIE. Based on Thompson and Sarnat scores, the infants treated during the second period had milder HIE than the infants treated during the first years after the implementation of TH in our hospital. Further research is necessary to evaluate the value and safety of TH for infants with mild HIE.

COI: None declared
ID: 533

TITLE: PROBABILISTIC GRAPHICAL MODEL IDENTIFIES CLUSTERS OF EEG PATTERNS IN RECORDINGS FROM NEONATES

AUTHORS: Julia Winter 1, Alex Sarishvili 2, Heiko J. Luhmann 3, Eva Mildenberger 1

AFFILIATIONS: 1 Department of Neonatology, University Medical Center of the Johannes Gutenberg University Mainz, Germany; 2 Fraunhofer Institute for Industrial Mathematics, Kaiserslautern, Germany 3 Institute of Physiology, University Medical Center of the Johannes Gutenberg University Mainz, Germany

CONTENT:

Brain function monitoring in neonates during their stay in the neonatal intensive care unit (NICU) may be a valuable tool to evaluate brain development and to investigate factors interfering with it.

To simplify the information complexity presented in the EEG signal and in order to visualize clinically relevant features, we present a novel method for objective and automated quantitative EEG analysis. It constructs a complex probabilistic graph (Chow-Liu tree) (inter-channel-frequency-band dependency structure) from a given multi-channel EEG recording, in order to estimate the different generic neonatal brain states.

By applying the Chow-Liu method to the analysis of EEG recordings, all characteristics of the EEG signal and their interdependencies can be displayed in a graphical model.

We tested the analytic algorithm by using retrospective EEG recordings of 28 neonates (postmenstrual age 37 – 44 weeks; 7 preterm). 23 recordings had been interpreted by a pediatric neurologist as normal (5/23 infants had chronic diseases). 5 recordings of infants with neurologic diseases were pathologic.

We computed the distances between trees (sum of tree edits operations, i.e. removing edge and adding edge operations until translation of one tree into another tree). The tree edit operations were weighted by the corresponding estimated mutual information. The trees were embedded into a 3-dimensional Euclidean space.

Using this approach, we were able to identify clusters of physiological and pathophysiological EEG patterns. The algorithm merged the 28 EEG recordings into 6 mathematically optimal clusters (see figure).

The method was able to select 4 of the 5 pathologic EEG recordings from the 23 recordings interpreted as normal (red cluster). Regarding the distances of cluster centers, the clusters comprising the majority of normal EEG recordings were close together (blue, magenta, cyan cluster). The group of pathological recordings showed the highest distance to these clusters.

The method identified also other clusters of EEG recordings that apparently had similar structures: we found similarity in EEG recordings of a group of infants with chronic diseases and a preterm infant (black cluster) and the cluster to which the algorithm merged the fifth pathological recording (yellow cluster).

Our method contrasts to all methodological approaches applied so far, as it considers differences / similarities between individual EEG recordings in order to cluster them in a mathematically derived optimal manner. The system works as a self-learning system.

The method may provide a basis for the future development of a non-invasive brain monitoring tool which will be able to differentiate between varieties of complex clinical findings.

IMAGES:
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Figure: The distances between Chow-Liu trees embedded into the 3d Euclidean space: identification of 6 clusters

COI: None declared
**ID:** 655  
**TITLE:** CORRELATION OF SERUM CYTOKINES TO NEUROIMAGING IN NEONATAL ENCEPHALOPATHY  
**AUTHORS:** M O'Dea1-6,8 T Hurley 1-3,6-8, L Kelly1,6,8, T Strickland6,8, A Byrne9, EJ Molloy1-9  
**AFFILIATIONS:** Coombe Women and Infant's University Hospital, Dublin, Ireland  
Rotunda Hospital, Dublin, Ireland; National Maternity Hospital, Holles St, Dublin, Ireland; National Children’s Research Centre, Ireland; National Children's Hospital Foundation, Dublin, Ireland; Trinity Translational Medicine Institute, Dublin, Ireland; Clinical Research Development Ireland, Dublin, Ireland; Department of Paediatric and Child Health, Trinity College Dublin, Ireland; Our Ladies Children’s Hospital, Crumlin  

**CONTENT:**  
Neonatal Encephalopathy (NE) describes central nervous system dysfunction in the earliest days of life in a term neonate. Inflammation is implicated in NE, it is known to have beneficial effects in recovery post-brain injury — however, when chronically dysregulated, it may lead to poorer outcome. We evaluated whether the inflammatory phenotype is predictive of abnormal neuro-imaging in NE, as an early serum biomarker before the MRI brain may be helpful to predict outcome.

Infants with NE Sarnat Grade II/III (n=25) undergoing therapeutic hypothermia were recruited. Serial serum samples were taken on days 1-4 of life. Multi-plex ELISA was performed on Interferon gamma (IFN-γ), Interleukin-1beta(IL1-β), Interleukin-1alpha(IL-1α), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-8 (IL-8) Granulocyte-macrophage colony stimulating factor(GM-CSF) tumour necrosis factor alpha(TNF-α), tumour necrosis factor beta (TNF β), Interleukin18 (IL-18), Vascular endothelial growth factor (VEGF), Interleukin 10 (IL-10), Interleukin-1 Receptor antagonist (IL-1RA), and Erythropoietin (EPO). MRI results were independently scored by a Paediatric Radiologist blinded to clinical outcome with the Barkovich scoring system. Cytokine results were correlated to Barkovich score.

11 patients had abnormal neuroimaging in this cohort. IL-1 α, IL-2, IL-6, IL-10, IL-18, IL-8, IL-1RA, IFN- γ, EPO, GMCSF, TNF-α, TNF-β levels did not differentiate between normal and abnormal neuroimaging. Higher IL-1β predicted an abnormal Barkovich score. Diagnostic accuracy of the test measured by the area under the ROC curve (AUC) was 0.81 (95% CI .69 to .93). The optimal cut-off value of 1.06pg/ml of IL-1beta had 100% sensitivity & 68% specificity for diagnosing abnormal MR brain.

Early raised serum IL-1β is predictive of abnormal neuroimaging. IL-1 β is known to be a pro-inflammatory cytokine that can produce injury to white matter in the developing brain. Il-1β shows promise for an early serum biomarker and a potential immunomodulatory target in NE.

**COI:** None declared
ID: 700

TITLE: PREVALENCE AND PRESENTATION OF NEONATAL-ONSET GENETIC EPILEPSIES

AUTHORS: Marie-Coralie Cornet 1, Anne Slavotinek 1,2, Dawn Gano 1,3, Shannon Rego 2, Hannah Glass 1,3,4, Donna Ferriero 1,3, and Roberta Cilio 1,2,3

AFFILIATIONS: 1. Department of Pediatrics, University of California San Francisco, San Francisco, California, USA
2. Institute for Human Genetics, University of California San Francisco, San Francisco, California, USA
3. Department of Neurology, UCSF Benioff Children’s Hospital, University of California San Francisco, San Francisco, California, USA
4. Department of Epidemiology & Biostatistics, University of California San Francisco, San Francisco, California, USA

CONTENT:

Seizures are common in the neonatal period occurring in 1-3/1000 neonates. Genetic epilepsies are disorders in which seizures are a core symptom, resulting directly from a known or presumed genetic mutation. Genetic epilepsies may present in the neonatal period and distinguishing them from the large number of seizures resulting from acute brain insults (i.e. HIE, stroke, CNS infections) has important implications for treatment and prognosis. We aim to describe the prevalence and presentation of neonatal-onset epilepsies due to genetic mutations in a cohort of neonates monitored with video-EEG per ACGME recommendations.

All neonates and infants monitored in a single level IV Intensive Care Nursery over a 4.5-year study period (05/2014-12/2018) were screened for inclusion. The video-EEG and medical records of neonates with documented seizures were reviewed. Etiology was determined to be genetic based on clinical, EEG, MRI and laboratory testing. Genetic testing was undertaken at the clinician’s discretion, with the constraint of insurance reimbursement. One patient was diagnosed in the P3EGS (Program in Prenatal and Pediatric Genomic Sequencing) study which offers whole exome sequencing (WES) to underrepresented and minority patients.

Over the study period, 494 neonates were monitored with video-EEG and 106 had seizures (21%). Eleven neonates (10%) were diagnosed with neonatal-onset genetic epilepsy. For 7 infants, the genetic mutation was found (KCNQ2:4; KCNQ3: 1; PRRT2:1; BRAT1:1), while for the remaining 4, 2 had a negative exome, 1 had a negative infantile epilepsy panel and 1 declined testing. MRI was performed in 7 and unrevealing. For the remaining 4 (3 of whom had a confirmed KCNQ2/3 pathogenic variant), MRI was not deemed necessary for the diagnosis. Seizure semiology was focal tonic in neonates with epilepsy associated with mutations in KCNQ2, KCNQ3, and PRRT2 genes, and myoclonic in the patient with BRAT1 mutation. Among the neonates with presumed genetic etiology, one had inconsistent semiology, one had focal tonic seizures, one had unilateral alternating focal clonic seizures, and one had ictal apnea.

In this large cohort of neonates with EEG-proven seizures a substantial minority (10%) were diagnosed with neonatal-onset epilepsy due to a known or presumed genetic variant. 70% of neonates who underwent genetic testing had a causal variant for their disease, confirming that genetic testing is key in neonates with seizures that are not clearly explained by an acute etiology.

COI: None declared
ID: 812

TITLE: INTRACRANIAL HAEMORRHAGE IN TERM INFANTS UNDERGOING THERAPEUTIC HYPOTHERMIA

AUTHORS: Elisa Smit 1, Richard Lee-Kelland1, Sally Jary 1, Andrew Whitelaw 1, Frances Cowan 1, Marianne Thoresen 1,2

AFFILIATIONS: 1 Department of Neonatal Neuroscience, University Of Bristol, Bristol, United Kingdom
2 Department of Physiology, University of Oslo, Oslo, Norway

CONTENT:

The incidence of intracranial haemorrhage (ICH) in term infants with neonatal encephalopathy (NE) undergoing therapeutic hypothermia (TH) is not well documented. We postulate that infants undergoing TH are at risk of ICH due to traumatic delivery, asphyxia, hypocarbia, hypoxia and acidosis, and clinical factors related to NE (hypotension, seizures, liver impairment and coagulation disturbance). TH in itself followed by gradual rewarming may pose an additional risk for the development of ICH. Cranial ultrasonography (cUS) before, during and following TH and routine magnetic resonance brain imaging (MRI) allowed us to describe the incidence and risk factors for ICH in infants undergoing TH.

Observational study over an 8-year period in a tertiary neonatal unit, which acts as the regional cooling centre. Infants ≥36 weeks with moderate or severe NE undergoing TH according to the extended CoolCap cooling criteria (n=193) were included. With ethical permission, demographic and clinical variables were collected prospectively. All infants underwent regular cUS on days 1-4 and a brain MRI scan on median day 8; both were used to identify ICH. Post mortem results were reviewed for the presence of ICH in non-survivors. Mann-Whitney U test, t-test, and Fisher-Exact test were used to compare groups. Regression analysis was used to identify factors associated with ICH. Survivors were followed up with Bayley-III neurodevelopmental evaluation at 18-24 months of age.

Intracranial haemorrhage was present in 70 infants (36%) and the predominant patterns of ICH were: 16% intraventricular (IVH), 21% intraparenchymal, 56% subdural/subarachnoid, 3% cerebellar, 2% sub-galeal haemorrhage. Seventeen infants (9%) had more than one type of ICH. Vaginal birth (80% in ICH vs 43% in no ICH group) and coagulopathy (36% vs 19%) were associated with ICH. An amplitude integrated EEG pattern before cooling of continuous normal voltage with seizures was seen in 21% of infants with ICH vs 8% in those without ICH (p<0.001). Infants with IVH showed significantly more thrombocytopaenia (<50 x10^9/L) and required more inotropic support. They also had a significantly lower cognitive Bayley outcome. Coagulopathy was associated with intraparenchymal haemorrhage. Vaginal birth, higher cord pH, and renal impairment were associated with subdural and subarachnoid haemorrhage.

More than 1 in 3 cooled infants with moderate to severe NE showed at least one type of ICH on brain imaging or post mortem examination. Vaginal birth is a known factor implicated in ICH, which we confirmed in this study. Coagulopathy was the second factor associated with ICH. Infants developing IVH appeared to have had greater cardiovascular instability and periods of hypotension, as reflected by their increased need for inotropic support.

COI: None declared
ID: 901
TITLE: RETRIEVAL AND TRANSPORT OF COOLED INFANTS: 1 YEAR REGIONAL SERVICE ANALYSIS
AUTHORS: Paul Cawley 1, Katherine Fenlon 1, Laura Martin 1, Laura Bubb 1, James Tooley 1
AFFILIATIONS: 1. Newborn Emergency Stabilisation and Transport Team (NEST), St Michael’s Hospital, University Hospitals Bristol, UK

CONTENT:

Therapeutic Hypothermia (TH) initiated within 6 hours of birth reduces death & disability in infants with moderate or severe Hypoxic Ischaemic Encephalopathy (HIE). Effectiveness of TH initiated after 6 hours is less certain. In the UK, infants requiring TH are centralised. Our network criteria for TH include evidence of perinatal asphyxia plus encephalopathy on examination & Cerebral Function Monitoring (CFM); interpretation is dependent upon the referring centre. We provide intensive care transport and clinical advice for our level 1 & 2 units. We have increasingly requested referring units send CFM traces to us to aid our clinical advice. We aimed to audit our service over the past year.

We audited all infants retrieved for uplift of care from a level 1 or 2 unit, to a level 3 unit, for management of HIE. All level 1 & 2 units within our region are able to initiate active TH. We continue active servo-controlled TH during transport. 12 month audit period: April 2018 to March 2019.

We assessed time to dispatch, time for infant to reach target core temperature (33.0-34.0°C), reasons for delay (>60 minutes), & reasons for failure to reach target temperature within 6 hours. We analysed if our transport team were able to view CFM images prior to dispatch, & if this impacted on decision to uplift.

Sources included discharge summaries, transport medical records & our transport database. Analysis: linear regression, median average & Interquartile Range (IQR).

We uplifted 44 infants. Gestational ages 35-42 weeks. Median time to dispatch when at base 52 minutes (IQR 41 to 56). No correlation between time of referral & time to dispatch (R2= 0.02). We observed clustering of referrals at the start & end times of medical shift patterns. Median time to target temperature 213 minutes (IQR 138 to 313). Age at time of referral was positively correlated with time to reach target temperature (R2= 0.52, p<0.0001). Failure to reach target within 6 hours occurred in 8 infants (18%); contributing factors included meeting criteria for TH towards 6 hours of age (27%), use of passive cooling (27%) & local misinterpretation of CFM (18%).

CFM was reviewed prior to dispatch for 22 infants (50%). In 2 cases (9%) an abnormal CFM was incorrectly interpreted as normal by the referring centre, in both instances this directly impacted decision to cool. (See figure)

A significant number of infants requiring TH are outborn & need uplift to tertiary centres. Correct interpretation of CFM can be difficult, but is vital for treatment decisions. Transport teams & receiving centres are able to remotely aid in decision making & may offer ‘fresh eyes’ for CFM interpretation. This may be especially prudent in infants where TH is not being initiated.

IMAGES:
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Pie chart demonstrating proportion of infants with CFM trace sent to transport team prior to dispatch. Breakdown shows percentage misinterpreted at the referring centre.

COI: None declared
TITLE: HYPOTHERMIA FOLLOWING RESUSCITATION WITH HYDROGEN GAS AFTER HYPOXIA-REOXYGENATION INHIBITS CASPASE-3 EXPRESSION IN PREFRONTAL CORTEX IN A NEWBORN PIG MODEL

AUTHORS: Leonid Pankratov 1; Torkil Benterud 1; Ronnaug Solberg 1,2; Moses Paneiakh 3; Ola Didrik Saugstad 1

AFFILIATIONS: 1 Department of Pediatric Research, University of Oslo and Oslo University Hospital, Rikshospitalet, Oslo, Norway
2 Department of Pediatrics, Vestfold Hospital Trust, Tønsberg, Norway
3 Department of Pathology, State Pediatric Medical University, Saint Petersburg, Russia

CONTENT:

Birth asphyxia and hypoxic-ischemic encephalopathy (HIE) are burdens to society worldwide. Moderate therapeutic hypothermia after room air (RA) resuscitation reduces the neurologic sequelae of HIE in term newborns. Caspase-3 is a hallmark of apoptosis in neuronal death. Our research group has shown that caspase-3 mRNA in the corpus striatum is down-regulated after resuscitation with RA. Hydrogen (H2) increased neuronal survival and suppressed Caspase-3 activity in a rat model of neonatal asphyxia. We suggested that resuscitation with 2.0 % H2 in RA combined with whole-body hypothermia (HT) would inhibit the expression of Caspase-3 in the prefrontal cortex compared to with RA resuscitation.

Forty-nine newborn pigs were randomized and subjected to either severe hypoxia (n=43) or normoxia (Control group, n=6). Animals exposed to hypoxia were further randomized into 4 groups: 1) resuscitation with RA followed by normothermia (RA+NT); 2) resuscitation with RA followed by HT (RA+HT); 3) resuscitation with H2 followed by NT (H2+NT); 4) resuscitation with H2 followed by HT (H2+HT). The observation period for groups was 9.5 hours after the end of hypoxia. Paraffin slides of cortex were prepared and cleaved Caspase-3 antibodies (Cell Signaling Technology, UK) for immunohistochemistry. Histoscanner Pannoramic MIDI II and Pannoramic viewer (3DHistech Ltd, Hungary) were used for analysis of Caspase-3 expression in the cortex. Statistical analysis was performed using SPSS Statistics 21.

There was a tendency towards the augmented cellular expression of Caspase-3 positive cells in all groups of hypoxia-exposed animals compared to the control group. Caspase-3 expression was significantly lower in both group receiving 2% H2 resuscitation compared to the RA+NT group (H2+NT: 0.1±0.1 vs. RA+NT: 0.29±0.27, p=0.046) and (H2+NT: 0.07±0.04 vs. RA+NT: 0.29±0.27, p=0.021). There was non-significant towards reduced of Caspase-3 activation in the H2+HT compared with the RA+HT group (0.07±0.04 vs. 0.18±0.17, p=0.057). The analysis of Caspase-3 positive cells did not show significant differences between groups receiving H2 followed by normothermia and hypothermia.

The expression of Caspase-3 positive cells in the cortex was significantly decreased after resuscitation with 2.0% hydrogen gas followed by normothermia or in combination with whole-body hypothermia compared with room air resuscitation and normothermia. Our data indicate that resuscitation with hydrogen gas may have an anti-apoptotic effect in the cortex after severe asphyxia. Whole-body hypothermia could enhance the influence of hydrogen gas.

IMAGES: https://www.eiseverywhere.com/eselectv3/v3/events/351149/submission/files/download?fileID=2183d1661e08c34c3c35a1d861eb949c-MjAxOS0wNSM1Y2UyNjY2ZDQwNWM5

Expression of Caspase-3 positive cells

COI: I have no conflict of interest
ID: 987

TITLE: DIAGNOSTIC BRAIN MRI FINDINGS IN MASSIVE NEONATAL ARTERIAL ISCHAEMIC STROKE (M_NAIS). CLINICAL AND PROGNOSTIC CHARACTERIZATION.

AUTHORS: Gemma Arca 1; Juan Arnaez 2; Thais Agut 3; Malaika Cordeiro 4; Nuria Boronat 5; Isabel Benavente 6; Simón Lubián 7; Eva Valverde 8; Christian Núñez 9; Christian Stephan-Otto 10; Alfredo García-Alix 11.


CONTENT:

Neonatal arterial ischemic stroke (NAIS) is defined as acute symptomatic focal cerebral infarction in an arterial territory between birth and 28 days of life that is confirmed by neuroimaging. NAIS usually involve Median Cerebral Artery (MCA). Occasionally, MCA-NAIS is considered to be massive (M_NAIS). In adults, it is defined as malignant or catastrophic infarcts when the TAC shows a stroke involving more than 50% from the MCA. We questioned if there are massive infarcts in neonates and about the relationship between qualitative findings and the volume of NAIS. Further we examine the clinical presentation and prognosis of patients suffering from a possible M_NAIS.

Prospective observational multicentre study; six paediatric university hospitals in Spain. Forty-five neonates with MCA-NAIS more than 35 weeks gestational age between 2009-2019 were studied. Infants with massive oedema of an hemisphere characterized by missing extraxial space and deviation from the middle line, and/or ventricular collapse uni or bilateral within the 48h from the onset of symptoms were considered to have M-NAIS by MRI (DWI) within six days after delivery. The lesions were segmented with ITK-Snap software to determine their volume. Neurodevelopment was assessed at 24 months using the Bayley-III, Gross Motor Function Classification System (GMFCS), and Bimanual Fine Motor Function (BFMF).

15 neonates had a M_NAIS and 30 a NonM_NAIS. Clinical debut occurred at a median of 18 hours after delivery. All M_NAIS were located significantly in MCA-M1, pre-bifurcation 9/15(60%) vs 3/30 (10%) of neonates with NonM_NAIS. Eight (57%) neonates with M_NAIS showed the absence signal in PLIC and, 13% NonM_NAIS. All M_NAIS had a lesion in optic radiations and, 13/15 (86%) had pre-Wallerian degeneration ( mainly in thalamus) and neonates with NonM_NAIS 14/30 (43%) and 6/30 (20%) respectively; p<0.002. Eight (80%) neonates with M_NAIS develop microcephaly during the first-year vs two (8%) NonM_NAIS; p<0.001. Of 13 infants with M_NAIS, 85% had an adverse outcome. Recurrent seizures, an adverse outcome and cerebral palsy in neonates with M_NAIS vs NonM_NAIS was significant. Median the relative infarct volume (RIV) M_NAIS was 21.99% (10.43, 27.71) vs 5.05% (2.45, 8.46) NonM_NAIS; p=0.002.

In our cohort, neonates with qualitative image criteria of M_NAIS had volume >20% of the hemisphere and these neonates had worse neuroutcome. The characterization of M_NAIS by MRI findings in neonates might be relevant for the prediction of outcome and also to allow for the identification of patients that could benefit from neuroprotective or neuroregenerative strategies.

COI: None declared
ID: 990

TITLE: AUTOPHAGY IN THE HIPPOCAMPUS AFTER SEVERE HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN TERM HUMAN NEONATES

AUTHORS: Anita C. Truttmann 1; Julia Gubler2; Vanessa Ginet1,2; Julien Puyal 2

AFFILIATIONS: 1Clinic of Neonatology, Department of Women, Mother and Child University Hospital, Center and University of Lausanne, Lausanne, Switzerland.
2Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland

CONTENT:

Macroautophagy, an essential physiological degradation process, has been recently demonstrated to be enhanced and importantly involved in neuronal death (by pharmacological or genetical means) occurring in hypoxic/excitotoxic and apoptotic conditions in vitro and in vivo rodent models. Moreover, autophagy has been shown to be increased in the thalamus and basal ganglia of died human newborns presenting severe hypoxic-ischemic encephalopathy (HIE). The present study examines whether neuronal autophagy is also enhanced and related to neuronal death processes in the hippocampus of asphyxiated human newborns.

Human hippocampal samples were obtained from at least 10 autopsied human newborns selected retrospectively from death reports of the clinic of Neonatology (Lausanne University Hospital, Switzerland) between 2001-2015. The criteria for selection of HIE cases were: gestational age >36 weeks, diagnosis of perinatal asphyxia (Apgar<5 at 5 minutes, arterial pH<7.0 at 1 hour of life and clinical encephalopathy Sarnat III). The brains of 5 HIE and 5 control (comparable gestational age dead from other conditions such as cardiopathy or diaphragmatic hernia) cases were analyzed. Neuronal autophagy was evaluated on histological sections by immunohistochemistry against autophagosomes (LC3) and lysosomal (LAMP1, cathepsins) markers in different hippocampal regions (CA1, CA3 and dentate gyrus (GD)).

Immunohistochemistry against LC3 and quantification of the number of LC3-positive dots per µm² showed that the number of autophagosomes increased significantly in all the hippocampal regions investigated, i.e. GD (by 9.36 fold), CA1 (by 7.24 fold) and CA3 (by 6.3 fold) in HIE compared to control cases. Since enhanced autophagy flux is associated with an increased presence of autolysosomes, which are larger than lysosomes, the number and size of CATHD- or LAMP1-positive dots were analyzed and quantified. The number and size of CATHD- and LAMP1-positive vesicles were significantly increased in the 3 regions investigated (DG, CA1 and CA3) in HIE compared to control cases. All together, these results suggest that, following severe perinatal asphyxia, human hippocampal neurons display an enhanced autophagic flux in HIE cases.

These results suggest for the first time that autophagy is enhanced in severe HIE in dying hippocampal neurons of human newborns, confirming previous observations on thalamus and basal ganglia. HIE-enhanced autophagy appears to be widely involved in all the brain regions affected by perinatal asphyxia and is then an interesting target for the development of future neuroprotective strategies in such conditions.

COI: None declared