September 22nd, 2023 15:00 - 17:00

PARALLEL SESSION 25 - BRAIN 5

ID 302. CHARACTERIZATION OF CELL DEATH AFTER PERIVENTRICULAR HEMORRHAGIC INFARCTION IN PRETERM INFANTS

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BACKGROUND

Periventricular Hemorrhagic Infarction (PVHI) occurs in 5–10% of very preterm newborns (VPT, <28weeks) in the first week of life and carries substantial neurological morbidity To date no study has systematically investigated the different types of cell death and which cell type is affected in the context of PVHI. Understanding the way cells are damaged is crucial to design brain protective and/or repair strategies.

METHODS

Human brain tissues were obtained from 23 VPT (Institute of Pathology, Lausanne) with ethical approval. The studied population of VPT born <28 0/7 gestational age

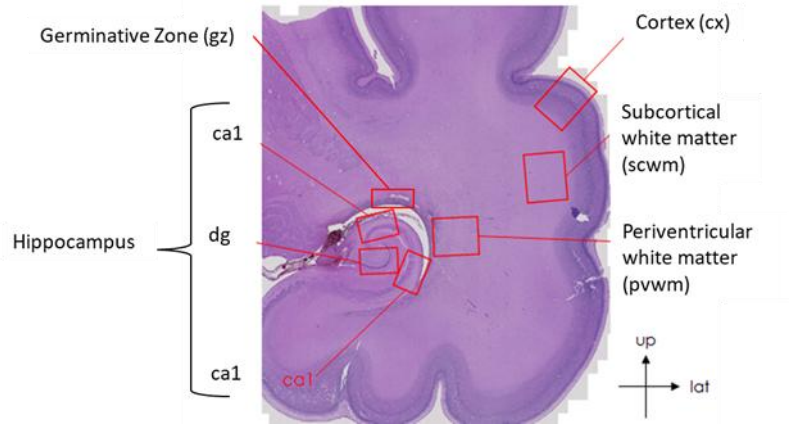
(GA) was selected retrospectively from death reports of the Clinic of Neonatology (CHUV, Lausanne) from 2005 to 2015. Cases with PVHI (ultrasonographic and pathological diagnosis, n=12) were compared to controls without significant brain injuries according to the autopsy reports (n=11). Newborns with cerebral malformations and known genetic anomalies were excluded. Haematoxyline–Eosine (HE) and Immunohistochemistry (IHC) stainings were performed with cell type and cell death markers in 5 different regions of interest (ROIs) (; see figure 1).

RESULTS

The mean GA at birth was 26 in both groups, without significant difference in birth weight or gender. In HE staining, we found a significant decrease in cortical thickness after PVHI, but no difference in global cellular density. In IHC, we found a significant increase in GFAP (astroglia), cCASP3 and TUNEL (apoptosis), pMLKL (necroptosis) and Iba1 (microglia) in the white and grey matter after PVHI. We also found a significant increase in LC3 positive dots (autophagosomes), CathD positive dots and dots mean area (autolysosomes) in the ipsilateral white and grey matter after PVHI, suggesting an increased autophagic flux.

CONCLUSIONS

A strong astrogliosis occurs in the overall brain of VPT after PVHI, and apoptotic and necroptotic markers are significantly increased. There is also an increased inflammation and indications of enhanced autophagy, suggesting its possible role in cell death after PVHI. We are now using markers of other types of cell death (ferroptosis, pyroptosis) as well as performing co-labelling analysis of cell death and cell type markers to characterize the type of dying cells in every brain region after PVHI in immature brains.



None declared

ID 312. EARY PHENOBARBITONE TREATMENT REDUCES SEIZURES INDUCED INCREASE IN CEREBRAL PERFUSION IN NEWBORN BRAIN

Alan Bainbridge[1], Magdalena Sokolska[1], Francisco Toarrealdea[1], Alexiane Touze[2], Kelly Harvey–Jones[3], Vinita Verma[3], Chris Meehan[3], Alison Mintoft[3], Ellie Campbell[3], Katie Tucker[3], Frederic Lange[4], Ilias Tachtsidis[4], Xavier Golay[2], Geraldine Boylan[5], Nicola Robertson[3], **Doctor Subhabrata Mitra**[3]

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Background

Seizures induce changes in cerebral metabolism and perfusion. MR imaging and proton MR spectroscopy (1H MRS) are standard MR modalities used for clinical assessment. Arterial spin labelling (ASL) MRI and phosphorus (31P) MRS are other useful tools for the assessment of cerebral oxidative metabolism and perfusion. The objective of this study was to comprehensively assess the impact of neonatal seizures on brain perfusion and metabolism using MR imaging, ASL MRI, 1H MRS, and 31P MRS in a preclinical model.

Methods

Ten healthy male newborn piglets underwent seizure induction with bicuculine 4mg/kg IV over two time points 4h apart. Another seven piglets had similar seizure induction and treatment with phenobarbitone 20mg/kg IV after 10min of continuous electrographic seizures, after each induction.

Piglets were sedated and anaesthetised throughout the experiment and underwent EEG and systemic monitoring. Imaging data were acquired in a 3T scanner. Each animal had a scan at the start of the study and a repeat scan after 10 hours.

ASL was performed using pseudo–continuous labelling (pCASL). Cerebral blood flow [ml/100g/min] maps were computed and mean CBF within regions was quantified using an automated atlas segmentation approach. Chemical shift imaging (CSI) was acquired for 1H MRS. 31P MRS was acquired with Image–Selected In vivo Spectroscopy (ISIS), adapted to include the whole brain.

Results

Generalised tonic clinic seizures with corresponding electrographic changes were noted immediately after bicuculine administration.

Significant differences were noted between the pre–ictal and post–ictal period in the whole brain (P=0.007) and cortical (P=0.004) cerebral blood flow (CBF) (figure 1A,1B).

Following phenobarbitone treatment, CBF reduced significantly in the post–ictal period in the whole brain (P=0.0072), cortex (P=0.0146,) and DGM (P=0.0342,) (figure 1A,1C).

A trend towards an increase in intracellular pH (pHi) was noted in the post–ictal period (7.13±0.07 vs 7.18±0.08), with a reduction in brain high energy phosphates (NTP/epp). No significant differences were noted following treatment. Deep grey matter Lac/NAA (lactate/N acetyl aspartate ratio) did not differ between the groups.

Conclusion

Increased whole brain and cortical perfusion persisted even 6 hours after the seizures (untreated). Early phenobarbitone treatment had a significant impact in reducing the seizure–induced increased perfusion.

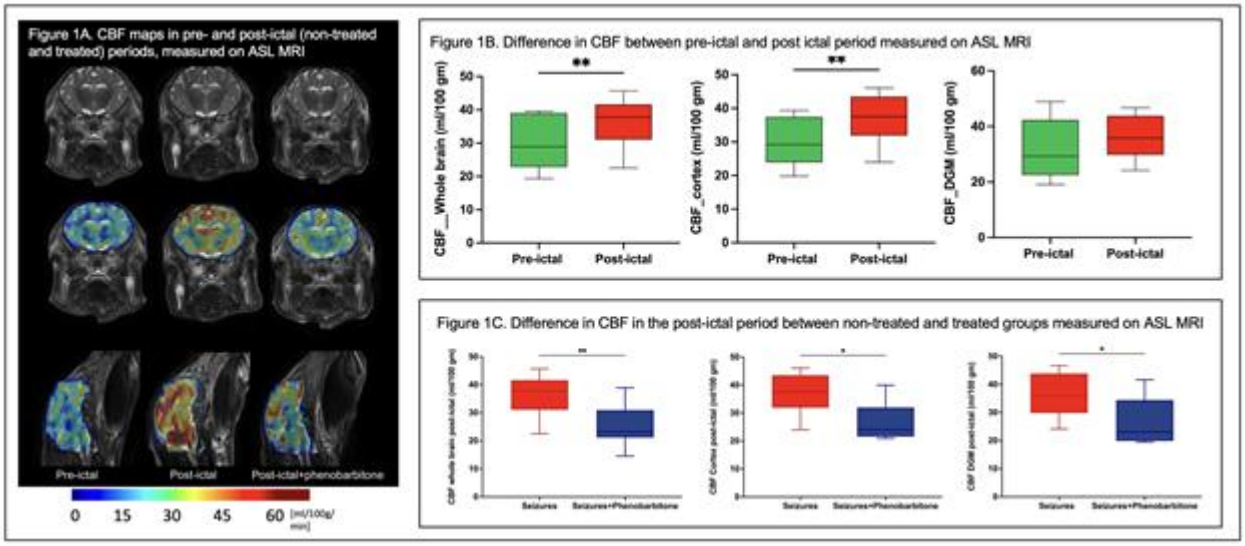

Figure 1. Increased whole brain (P=0.007) and cortical (P=0.004) perfusion were noted following seizures (figure A&B). Early phenobarbitone reduced CBF in the whole brain (P=0.0072), cortex (P=0.0146) and DGM (P=0.0342).

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None declared

ID 239. Extracellular vesicles from immortalized and clonally expanded mesenchymal stromal cells as adjunct therapy to therapeutic hypothermia for neonatal hypoxic-ischemic brain injury

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Background

Neonatal encephalopathy (NE) caused by hypoxia-ischemia (HI) is a leading cause for childhood morbidity and mortality. The only available but obligatory therapy is hypothermia (HT), which is, however, limited due to a short therapeutic window. Despite tremendous research, an adjuvant therapy overcoming limitations of HT is still missing. Extracellular vesicles (EVs) from mesenchymal stromal cells (MSCs) showed promising effects in different neonatal and adult brain injury models. According to issues associated with MSC heterogeneity and senescence, we recently demonstrated a high therapeutic efficiency of EVs from immortalized and clonally expanded MSCs (ciMSCs). In the present study we investigated, whether an intranasal ciMSC-EV therapy overcomes limitations of HT.

Methods

Nine-day-old C57BL/6 mice were exposed to HI through ligation of the right common carotid artery and 1 hour hypoxia (10% oxygen) followed by 4 hours hypothermia or normothermia. ciMSC-EVs were administered intranasally at day 1, 3

and 5 after HI. Analyses of brain tissues were performed via immunohistochemistry, western blot and real time PCR at day 7 post HI. Long-term functional outcome was assessed by neurobehavioral testing 35 days after HI.

Results

Confirming previous reports, therapeutic effects of HT were limited with regard to protection in the striatum and improvement of myelination deficits. Furthermore, long-term functional outcome was only partially improved. Compared to a HT single therapy, the combined treatment with ciMSC-EVs resulted in an increased protection from HI-induced neuronal loss, astro- and microgliosis and myelination deficits, accompanied by an increased neurotrophic growth factor expression. These short-term effects translated into long-term improvement of cognitive deficits and amelioration of HI-induced alterations in risk assessment behavior.

Conclusion

The availability of ciMSCs provides new avenues for the standardized and scaled manufacturing of clinical grade EV products. Importantly, the present findings demonstrate that intranasal administration of ciMSC-EVs improve the outcome of HT therapy. Therefore, ciMSC-EV application appears a promising adjuvant therapy, overcoming current limitations of the obligate clinical care for the treatment of NE caused by HI.

None declared

ID 498. INTRAVENTRICULAR LEVELS OF CELL-FREE HAEMOGLOBIN AND SCAVENGING PROTEINS FOLLOWING SEVERE CEREBRAL INTRAVENTRICULAR HAEMORRHAGE IN EXTREMELY PRETERM INFANTS

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Background

Cerebral intraventricular haemorrhage (IVH) is a major cause of neuro-developmental impairment in preterm infants. Cell-free haemoglobin (Hb), released into the intraventricular space due to erythrocyte lysis, is a causal initiator of pro-inflammation, oxidative stress and tissue damage. Endogenous detoxifying and scavenging proteins include haptoglobin and hemopexin. Our preclinical studies have shown that intraventricular administration of haptoglobin decreases inflammation and prevents tissue damage following IVH. Potential translation of this treatment paradigm to preterm infants requires knowledge of cell-free Hb kinetics and of intrathecal concentrations of endogenous haptoglobin and hemopexin in intraventricular CSF following IVH.

Methods

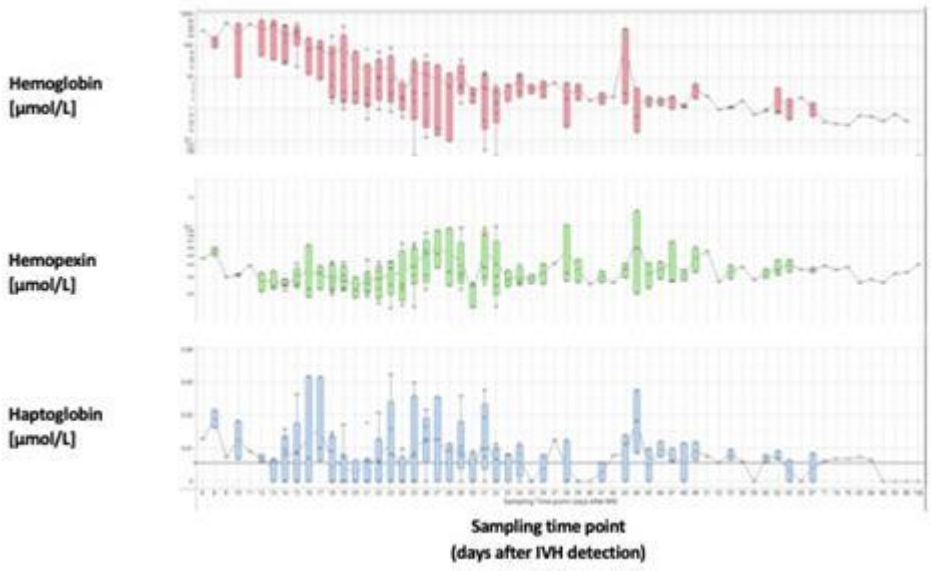
Prospective observational study at a level III NICU, Skåne University Hospital, Lund, Sweden. 16 extremely preterm infants with a mean (SD) gestational age at birth of 27.2 (2.7) weeks and birthweight 1042 (369) g. All infants developed either IVH grade III (N=11) or periventricular haemorrhagic infarction (N=5) at a mean (SD) postnatal age of 3.1 (3.8) days. All infants received a neurosurgically inserted intraventricular reservoir at a mean postnatal age of 18 (11) days enabling longitudinal CSF withdrawal (approx. 10 ml/kg/day). Concentrations of cell-free Hb (spectrophotometry), haptoglobin and hemopexin (LC-MS/MS) were determined in 167 CSF samples corresponding to a mean (SD) number of 10.4 (11.6) samples per infant.

Results

Intraventricular CSF concentration of cell-free Hb decreased during a 3-week period from a median (range) of 461 (1.5—511.3) µmol/L at time-point of insertion of reservoir to 0.4 (0.1—12.0) µmol/L (figure 1). Concentrations of haptoglobin and hemopexin were extremely low and did not vary over time (figure 1).

Conclusion

Intraventricular levels of cell-free Hb decreased exponentially during the first 3 weeks following cerebral IVH. Levels of haptoglobin and hemopexin in intraventricular CSF were extremely low throughout the study period indicating a severe deficit in endogenous cell-free Hb protection resources. These novel data provide an initial basis for calculation of the amount of intrathecal haptoglobin needed to scavenge the cell-free Hb following severe IVH.



Longitudinal levels of cell–free hemoglobin, haptoglobin and hemopexin in cerebrospinal fluid following severe intraventricular haemorrhage in very preterm infants. Boxplots depict medians, first/third quartile. Whiskers depict minimum and maximum.

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VV, SMP, KG, TG and MB are employees of CSL Behring.