September 17th, 2021 15:00 - 17:00

PARALLEL SESSION 26 - BRAIN 4

ID 412. OBSTRUCTIVE CHOLESTASIS COMPROMISES BALANCE AND ALTERS BRAIN LIPID COMPOSITION IN NEONATAL PIGLETS

Miss Nicole L. Henriksen[1], Mr Svend H. Hansen[2], Mr Matthew D. Lycas[3], Miss Xiaoyu Pan[1], Mr Thomas Eriksen[4], Mr Lars S. Johansen[5], Mr Christer Ejsing[6], Mr Douglas G. Burrin[7], Mrs Vibeke B. Christensen[8], Mr Thomas Thymann[1], Mrs Stanislava Pankratova[1]

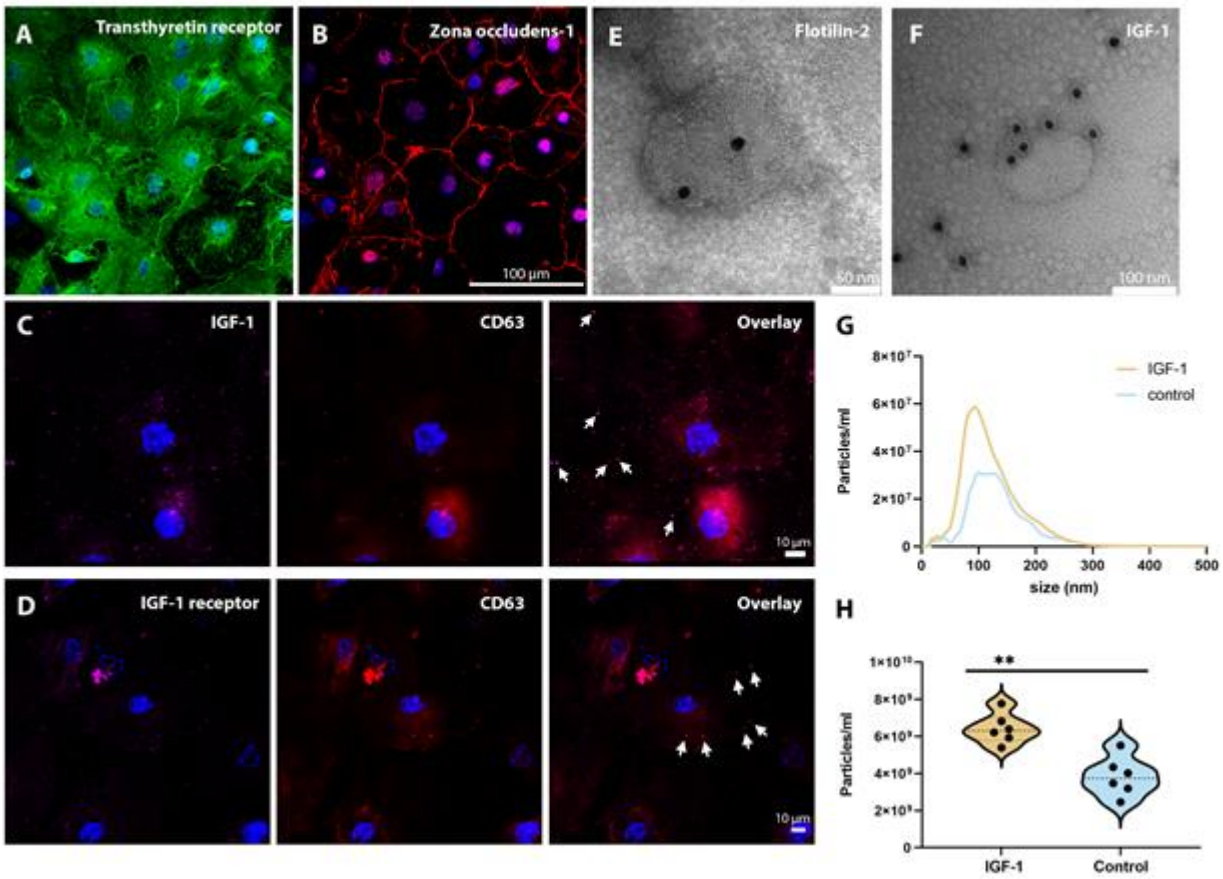
[1]Comparative Pediatrics and Nutrition, Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark, [2]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark, [3]Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark, [4]Department of Veterinary Clinical Sciences, University of Copenhagen, Copenhagen, Denmark, [5]Department of Pediatric Surgery, Rigshospitalet, Copenhagen, Denmark, [6]Department of Biochemistry and Molecular Biology, VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, Odense, Denmark, [7]Department of Pediatrics, United States Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, Baylor College of Medicine, Houston, USA, [8]Department of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen, Denmark

BACKGROUND: Infants with neonatal cholestasis are prone to neurodevelopmental deficits including neuromotor function. They accumulate potentially neurotoxic molecules in the bloodstream including ammonia, bilirubin and bile acids, and are at risk of malabsorbing lipids important for brain development. This study examined neuromotor function and bile acid and lipid composition of the brain in a piglet model of neonatal obstructive cholestasis.
METHODS: Eight-day old piglets underwent bile duct ligation (BDL) or sham surgery (SHAM) and were followed for three weeks during which neuromotor functional tests (balance beam, open field, neuromotor score, gait analysis) were conducted. Samples were collected for liver histopathology, RNA sequencing of the cerebellum, and bile acid and lipid profiling of the plasma and/or cerebellum.
RESULTS: BDL piglets had increased liver enzyme levels, liver fibrosis and bile duct proliferation compared to SHAM piglets. Balance was compromised in BDL piglets ($p<0.05$) but only minor changes were found in other tests of neuromotor function. Plasma and cerebellum bile acid profiles differed between BDL and SHAM piglets with hyocholic acid and conjugated bile acid forms dominating in the BDL group (approximately 89% and 99% of total bile acids, respectively). Total bile acids and levels of the bile acid biosynthesis marker C4 (7α-hydroxy-4-cholesten-3-one) in plasma and cerebellum were increased in the BDL group, whilst plasma FGF-19 levels were reduced (all $p<0.001$). In the cerebellum there were lower total lipid, phosphatidylinositol and 18:2 fatty acid levels, while of several low abundance saturated and unsaturated fatty acids of both even and odd chain length were increased in the BDL group relative to SHAM (all $p<0.05$). RNA sequencing revealed similar gene expression profiles between groups.
CONCLUSION: In conclusion, surgically induced neonatal obstructive cholestasis compromised aspects of neuromotor function and altered the bile acid and lipid profile of plasma and/or cerebellum in piglets. Longer-term studies are needed to determine these effects at a stage of more advanced liver disease.
None declared

ID 432. INSULIN GROWTH FACTOR 1 STIMULATED RELEASE OF EXTRACELLULAR VESICLES FROM THE CHOROID PLEXUS – NOVEL MODE OF BLOOD-BRAIN-BARRIER SIGNALLING

Mr Niklas Ortenlöf[1], Miss Suvi Vallius[1], Ass Professor Magnus Gram[1], Professor David Ley[1]
[1]*Pediatrics, Department of Clinical Sciences Lund, Lund University, Lund, Sweden*

Background: It is well accepted that insulin-like growth factor 1 (IGF-1) plays a crucial role in brain development. However, previous studies have inferred that the transfer of systemically administered IGF-1 over the blood brain barrier is restricted, which would present a limitation for beneficial treatment effects within the brain. Extracellular vesicles (EVs) are small, cell-derived phospholipid membrane enclosed vesicles that constitute important cell-to-cell messengers, regulating diverse cellular functions of recipient cells. EVs released from the choroid plexus (CP) to intraventricular cerebrospinal fluid (CSF) have been shown to cross the ependyme and exert a regulatory function in periventricular brain tissue. We hypothesized that exposure of the CP to blood-born IGF-1 induces release of EVs into intraventricular CSF destined to regulate surrounding brain parenchyma. We evaluated this in a primary culture of CP epithelial (CPE) cells using a Transwell in vitro model.
Methods: CPE were collected from p3-p9 mice pups and plated on Transwell membranes. Cells were stimulated with 40 ng/ml IGF-1 at the basal (blood) side for 24 h, apical supernatant was collected, and cells were fixed. EVs were prepared from cell culture supernatant. The size and quantity of EVs were measured with nanoparticle tracking analysis. EV morphology and protein immunolabeling were evaluated with transmission electron microscopy (TEM). CP protein markers were analysed using immunocytochemistry.
Results: Functional characteristics of CPE cells were ascertained by positive staining for the transthyretin receptor and zona occludens-1 (Fig.1a,b), absent staining for several fibroblast markers and an increasing transepithelial electric resistance during culture. The CPE cells also stained positively for IGF-1, the IGF-1 receptor and the exosomal marker CD63 (Fig.1c,d). TEM of purified EVs from apical supernatant displayed typical exosome and microvesicle morphology, positive immunolabeling for IGF-1 and the exosomal marker Flotilin-2 (Fig.1e,f). Exposure of CPE cells to IGF-1 caused an increased release of EVs into the apical supernatant ($p = 0.004$) (Fig.1g,h).
Conclusion: Simulated blood-borne exposure of IGF-1 stimulates CP epithelial cells to secrete IGF-1 positive EVs in a Transwell in vitro system. We will further expand this study to investigate the miRNA secretome of CP derived EVs upon IGF-1 stimulation, both in vitro and in vivo.



(a-d) Immunocytochemical staining of the transthyretin receptor, zona occludens-1, IGF-1, the IGF-1 receptor and CD63. Arrows display colocalization. (e,f) TEM; immunogold labelling of Flotilin-2 and IGF-1. (g,h) Nanoparticle tracking analysis.

(a-d) Immunocytochemical staining of the transthyretin receptor, zona occludens-1, IGF-1, the IGF-1 receptor and CD63. Arrows display colocalization. (e,f) TEM; immunogold labelling of Flotilin-2 and IGF-1. (g,h) Nanoparticle tracking analysis.

None declared

ID 372. THE IMPACT OF INCREASED MATERNAL SFLT-1/PLGF RATIO AS A BIOMARKER FOR PREECLAMPSIA ON THE MOTOR OUTCOME OF PRETERM INFANTS

Ms Lisa Middendorf[1], Ms Alexandra Gellhaus[2], Ms Anne-Kathrin Dathe[1], Mr Ivo Bendix[1], Ms Beatrix Reisch[2], Ms Ursula Felderhoff-Mueser[1], Ms Britta Huening[1]

[1]University Hospital Essen, Department of Pediatrics I, Essen, Germany, [2]University Hospital Essen, Department of Gynecology and Obstetrics, Essen, Germany

Background: The sFlt-1 (soluble fms-like tyrosine kinase-1)/PlGF (placental growth factor) ratio serves as a biomarker to predict preeclampsia. Elevated levels also increase the risk for prematurity and intrauterine growth restriction. However, its predictive value for subsequent neurological outcome has not yet been examined. This study aims to evaluate the correlation between maternal sFlt-1/PlGF ratios and early motor outcome of preterm infants.

Methods: 82 preterm infants (gestational age ≤ 34+4) born between February 2017 and August 2020 at the Department of Obstetrics and Gynecology, University Hospital Essen, Germany, were included. The following variables were available: maternal sFlt-1 and PlGF levels and general movement assessment (GMA) of the infant at the corrected age of 10-16 weeks. The infants were stratified into high and low ratio groups according to maternal sFlt-1/PlGF cut off of 85. To investigate the early motor repertoire and quality of spontaneous movements of the infant, the Motor Optimality Score (MOS-R) based on antigravity movements and posture patterns, was applied. This approach allows for early identification of severity and type of neurological dysfunctions (Einspieler et al, 2019).

Results: Linear regression analysis shows that the sFlt-1/PlGF ratio does not predict the MOS-R score (β= -0,09; p= 0.424). However, children with birth weight below the 10th percentile scored significantly lower (mean 21.0 vs 22.7; p=0.05). These children were in 84% in the group with an increased sFLT1/PLGF ratio, which in turn is a predictor of low birth weight (β=-0,389; p=<0.001).

Conclusion: In this cohort, low birth weight correlates with an elevated sFLT-1/PLGF ratio and had a negative effect on the outcome in the MOS-R. A direct correlation between an increased ratio and a worse motor outcome was not demonstrated.

None declared

ID 186. Exposure to fetal growth restriction and consequence on neonatal microglia proteome in rat

PhD Manuela Zinni[1], Engineer Julien Pansiot[1], PhdMD Marina Colella[1], Engineer Valerie Faivre[1], PhD Jerome Mairesse[2], PhdMD Andrée Delahaye-Duriez[1,3], PhD Daniel Vaiman[4], Professor Olivier Baud[1,2,5]

[1]Inserm U1141, Paris, France, [2]Laboratory of Child Growth and Development, University of Geneva, Geneva, Geneva, Switzerland, [3]UFR de Santé, Médecine et Biologie Humaine, Université Paris 13, Sorbonne Paris Cité, F-93000 Bobigny, France, Bobigny, France, [4]Institut Cochin, Inserm U1016, UMR8104 CNRS, PARIS, France, [5]Division of Neonatology and Pediatric Intensive Care, Children's University Hospital of Geneva, Geneva, Switzerland., Geneva, Switzerland

Background
Intra-uterine growth restriction (IUGR), is a leading cause of ante/perinatal stress and brain injury, responsible for neurocognitive and behavioral disorders. Perinatal inflammation and the consequent microglia activation are key factors of brain vulnerability in neonates born with IUGR. However the consequences of IUGR on microglia development and on microglia proteome are still unknown. The aim of this study was thus the characterization of microglia proteome in response to IUGR and the description of the effect of IUGR on microglia proteome during development. In addition a concordance analysis between proteome and transcriptome was performed.
Methods
We used a rat model of IUGR induced by a gestational low-protein diet (LPD). Microglia cells were magnetically sorted from control and LPD animals at postnatal day 1 (P1) and 4 (P4) and a proteomic analysis was performed using the label free technology. Proteomic data were analyzed using both ORA and GSEA analysis and additional biochemical approach was used to confirm proteomic results.
Results:
Significant changes of microglia proteome were observed early after birth in growth-restricted pups previously exposed to gestational LPD. Expression of protein sets associated with fetal growth were significantly enriched in LPD microglia at both P1 and P4. An upregulation of protein sets associated to inflammation and immune response was observed confirming the pro-inflammatory effect of LPD on microglia cells. Interestingly, an upregulation of protein sets associated with oxidative stress response and to ROS production was observed only at P4. This results were further supported by a exacerbated ROS production observed in primary microglial cell culture. During the development, inflammation-associated proteins were found to be upregulated between P1 and P4 both in control and LPD microglia. In contrast, proteins associated with DNA repair and senescence pathways were upregulated only in LPD microglia. Similarly protein sets involved in the protein retrograde transport were significantly downregulated only in LPD.
Conclusions:
Overall, these data demonstrate significant effect of LPD-induced IUGR on developmental program of microglial cells leading to abnormal proteome within the first postnatal days.

None declared