



September 17th, 2021 08:30 - 10:30

PARALLEL SESSION 20 - BRAIN 5

ID 480. SYSTEMIC IGF-1 TREATMENT AFFECTS GENE EXPRESSION IN THE BRAIN IN THE PRETERM RABBIT

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Background: Endogenous serum levels of IGF-1 are low in preterm infants following birth, and low levels are linked to morbidities and unfavorable neurodevelopmental outcome. IGF-1 affects brain cell proliferation, neurogenesis, maturation, and differentiation, however, the mechanistic effects of systemic exogenous IGF-1 on the brain, across the blood barrier still remain unclear. Using the preterm rabbit pup as a model for preterm infants, we investigated the impact of systemic peripheral IGF-1/IGFBP3 treatment on gene expression in the periventricular brain area.

Methods: Preterm rabbits (n=24) were delivered by C-section on E29 (=three days prior to term) and received 4 mg/kg IGF-1/IGFBP3 or NaCl subcutaneously every 12th hour, the first three days of life (6 doses). Total RNA transcriptome sequencing was performed on periventricular brain matter collected after the end of treatment (P0, 3 days after birth) and 10 days after the end of treatment (P10).

Results: IGF-1/IGFBP3 treatment resulted in significant changes in 1624 genes ($q \leq 0.05$, corrected for multiple testing) at P0 compared to the NaCl group. No differently expressed genes were found at P10. Over time, from P0 to P10, 1813 genes were affected exclusively by IGF-1/IGFBP3 treatment, 1583 genes were regulated exclusively in the NaCl group, and 1001 were affected in both groups. Top regulated KEGG pathways at P0 in the IGF-1/IGFBP3 treatment included the spliceosome, the AMPK and the Hedgehog signalling pathways, all involved in regulating brain cell proliferation, metabolism, stem cell maintenance, and development. Investigating the dynamics in gene expression from P0 to P10, top regulated pathways affected in the IGF-1/IGFBP3 group included the synaptic vesicle cycle, the cell cycle, and the GABAergic synapse pathways among others. The top 20 genes upregulated in the IGF-1/IGFBP3 group from P0 to P10 (not affected at P0) included SCN1B, HPCA, CNTNAP2 and ITPKA gene, critical for neuronal migration and proliferation, myelination and synaptic plasticity.

Conclusions: Systemic peripheral IGF-1/IGFBP3 treatment postnatally following preterm birth induced changes in genes and important pathways involved in neurodevelopment, such as the formation of neurons, neuronal survival, and synaptic plasticity. Further, IGF-1/IGFBP3 treatment triggered a different developmental cue affecting the dynamics of the gene expression during an important developmental period.

DL has received consulting fees from Shire PLC.



ID 152. MATERNAL MELATONIN ADMINISTRATION LEADS TO IMPROVED BRAIN MYELINATION IN GROWTH RESTRICTED FETAL LAMBS

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Background: Fetal growth restriction (FGR) is a serious pregnancy complication associated with increased risk of adverse neurodevelopment in the offspring. Melatonin can be safely given to the mother during pregnancy, and it readily crosses the placenta and fetal blood brain barrier. We investigated the effects of maternal melatonin administration on fetal brain structure in early-onset FGR, and assessed the presence and distribution of melatonin receptors, MT1 and MT2. Methods: Surgery was performed on twin-bearing pregnant ewes. Placental insufficiency and subsequent FGR was induced via single umbilical artery ligation in one of the twin fetuses at 88 days (0.6 gestation). Melatonin was administered intravenously (6 mg/ day) to a group of ewes from the time of surgery until 125 days (0.8 gestation), at which point the ewe and fetuses were euthanased, and fetal brains collected. Results: Study groups included control (n=5), FGR (n=5), control melatonin (control+MLT; n=6) and FGR melatonin (FGR+MLT; n=6). Melatonin administration did not alter fetal body or brain weights and was well tolerated. There were no significant differences seen in oligodendrocyte (Olig-2+) counts across all brain regions examined. Myelin (CNPase+) fibre density was reduced in FGR vs. control animals in most brain regions (p<0.05) and melatonin treatment restored CNPase fibre density. A similar but less pronounced trend was seen with mature myelin (MBP+). Significantly increased astrocyte (GFAP+) immunoreactivity was seen in the intragyral white matter and cortex of FGR vs. control animals, while melatonin decreased immunoreactivity in corpus callosum and external capsule (EC). Significant differences in activated microglia (Iba-1) activity were seen between FGR vs. FGR+ MLT groups in periventricular white matter (PVWM), subventricular zone and EC (p<0.001). MT1 receptors were found only in white matter, however no significant differences were noted between groups. MT2 receptors were increased in PVWM in FGR animals, and reduced in FGR+MLT animals. Conclusions: Maternal melatonin administration in an early onset model of FGR led to improved myelination of white matter brain regions, possibly mediated by decreased inflammation.

None declared



ID 279. SEVERE IVH WITH PHVD ALTERS BRAIN WHITE AND GRAY MATTER STRUCTURE, LEADING TO ABERRANT CORTICOGENESIS IN THE PRETERM RABBIT PUP

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Background: Up to 60% of infants with severe intraventricular hemorrhage (IVH) develop post-hemorrhagic ventricular dilatation (PHVD), resulting in neurodevelopmental impairment. There are scarce data available on long-term neurodevelopmental outcomes of IVH in animal models, limited mainly to term-born animals. We aimed to establish a long-term model of PHVD in preterm rabbit pups and characterize the pattern of white and gray matter injury, and cortical impairment.

Methods: IVH in preterm rabbit pups was induced by intraperitoneal injection of glycerol at postconceptional day 29 (full-term = 31). The presence of IVH was confirmed by high-frequency ultrasound at 24 h of age. The preterm rabbit pups were raised by a wet-nurse until postnatal day 33. Immunostainings were applied to investigate neurogenesis, synaptogenesis, astrogliosis, myelination, and corticogenesis. The assessment of the orientation and directionality of myelinated fibers was performed.

Results: The occurrence of IVH in the study was 58 % (45/77). Survival of pups with IVH/PHVD (17.8%; 8/45) was significantly reduced compared to control pups (40.6 %; 13/32). Twenty pups (IVH/PHVD = 7) were used for analysis. The pups with IVH/ PHVD had globally reduced myelin content compared to controls ($p = 0.0009$), an aberrant cortical myelination microstructure, and thinner upper cortical layers (I-III) (mean diff -3.31; 95%CI -6.53-(-) 0.09; $p = 0.04$). We observed lower number of parvalbumin (PV)-positive interneurons in deeper cortical layers (IV-VI) in IVH/PHVD animals (mean diff -4.28; 95%CI -8.25-(-) 0.31; $p = 0.03$).

IVH/PHVD inhibited the normal process of maturation of PV-positive interneurons, characterized by a reduction of the numbers of PV-positive perineuronal network-negative cells (mean diff -3.91; 95%CI -5.52-(-) 0.85; $p = 0.007$). Pups with IVH/PHVD had overall reduced neurogenesis and synaptogenesis compared to the controls ($p = 0.008$ and $p = 0.0003$, respectively). We observed signs of reduced microglial activation in pups with IVH/PHVD in all studied brain regions ($p = 0.08$) except the hypothalamus and internal capsule.

Conclusions: At one month of age, IVH/PHVD in the preterm rabbit pups resulted in alterations of cortical myelination microstructure and cortical organization with a reduction and maturational delay of PV-positive interneurons and a global decrease of neurogenesis and synaptogenesis.

None declared



ID 472. Preliminary results of the PASSIoN trial (Perinatal Arterial Stroke treated with Stromal cells IntraNasally)

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BACKGROUND: Perinatal arterial ischemic stroke (PAIS) is an important cause of perinatal brain damage in the term-born neonate with lifelong neurodevelopmental disorders in 50-75% of patients. Currently, there is no treatment available to alleviate neurological damage after PAIS. Mesenchymal stromal cells (MSCs) have shown promising results in animal studies of PAIS. In this study, we assessed the safety and feasibility of intranasal delivery of bone marrow-derived allogeneic MSCs in neonates with PAIS.

METHODS: We conducted a phase I/II, open-label, single-arm, nationwide intervention study in the NICU at the University Medical Center Utrecht, the Netherlands (ClinicalTrials.gov/show/NCT03356821). Ten (near-)term (≥ 36 weeks of gestation) neonates with MRI-confirmed PAIS in the middle cerebral artery (MCA) region, with presenting symptoms within the first week after birth and parental consent, were included. Neonates received one dose of $\pm 50 \times 10^6$ MSCs via intranasal droplets as soon as possible after confirmation of the MCA stroke, but within the first week after presenting symptoms. We monitored (sub)acute safety by measuring vital parameters, blood markers and occurrence of adverse events, and we repeated the MRI at three months of age.

RESULTS: In all ten neonates, intranasal administrations of MSCs were feasible. We did not observe any adverse events, except for one patient that developed a mild transient fever shortly after MSC treatment without further clinical implications. Blood infection parameters (CRP, procalcitonin and leukocyte levels) remained stable pre- versus post-administration. MRI scans at three months of age (n=8, 2 pending) did not show signs of infection or cerebral tumorigenicity and 63% (n= 5/8) of infants had minimal to no posterior limb of the internal capsule (PLIC) involvement while the corticospinal tract initially showed diffusion restriction on DWI. Visual inspection of the amount of tissue loss on MRI following MSC therapy looks promising, however quantitative analysis still needs to be performed. Currently, most neonates are too young to report on their functional outcome.

CONCLUSION: Intranasal MSC application of ten neonates with PAIS was safe and feasible. Most infants showed symmetrical myelination of the PLIC three months after MCA stroke. Future placebo-controlled studies with larger patient populations are needed to determine the therapeutic effect of MSCs.

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