Poster abstracts

P01

RESOLVIN D1 AMELIORATES LIPOPOLYSACCHARIDE-INDUCED BLOOD-BRAIN BARRIER DISRUPTION

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Sepsis is a life-threatening condition triggered by overreaction of immune system in response to infection eventually leading to significant morbidity and mortality. The resultant widespread inflammation and migration of immune cells to the brain, accompanied with increase of cytokines in the brain parenchyma, causes destruction of tight junctions (TJs) between barrier-type brain capillary endothelial cells leading to enhancement of blood-brain barrier (BBB) permeability through paracellular pathway. Resolvin D1 (RvD1), a lipid mediator derived from docosahexaenoic acid, displays potent anti-inflammatory and antioxidant activities. This study aimed to evaluate the effects of RvD1 against lipopolysaccharide (LPS)-induced inflammatory model of mouse brain endothelial cell line (bEnd.3). For this purpose, bEnd.3 cells cultured on inserts in transwells were treated with LPS (500ng/mL) for 24 hours followed by RvD1 (80µg/mL) for 24 hours, and trans-endothelial electrical resistance (TEER) and permeability of sodium fluorescein tracer was measured. In addition, immunofluorescent staining was performed to assess the immunoreactivity of claudin-5, a major TJ protein. The administration of RvD1 following LPS significantly increased the decreased TEER values and significantly decreased the increased passage of sodium fluorescein into the lower compartment of transwells (P<0.01). In addition, the immunostaining intensity of claudin-5 significantly decreased following LPS administration, while the subsequent RvD1 treatment significantly enhanced the decreased claudin-5 immunoreactivity (P<0.001). Our findings indicate that RvD1 ameliorates the enhanced permeability of bEnd.3 cells under LPS-induced inflammatory conditions by increasing the expression of tight junction protein, claudin-5, which suggests that this agent may account for a novel treatment modality in sepsis-induced BBB damage.

P02

ENDOTHELIAL OVEREXPRESSION OF ANGIOPOIETIN-2 EXERTS SEX-SPECIFIC EFFECTS ON CENTRAL NERVOUS SYSTEM AUTOIMMUNITY

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Multiple sclerosis (MS) is a complex neuroinflammatory disorder marked by blood-brain barrier (BBB) disruption, immune cell infiltration, and cytokine dysregulation. Among the elevated cytokines detected in MS and its animal model, experimental autoimmune encephalomyelitis (EAE), Angiopoietin-2 (Angpt- 2) stands out for its dual role in angiogenesis and inflammation. A role for Angpt-2 in EAE and potentially MS is proposed by the observation that functional inhibition of Angpt-2 ameliorates EAE. Employing a transgenic mouse model with inducible endothelial-cell specific overexpression of Angpt-2 we here show that elevated Angpt-2 levels result in vascular remodeling, inflammatory changes in the endothelium, as well as an increased accumulation of myeloid cells in peripheral tissues, while vascular permeability was not affected. Surprisingly, male but not female mice with endothelial overexpression of Angpt-2 showed ameliorated EAE when compared to control littermates. This sex-specific effect was associated with a pronounced accumulation of macrophages and T cells in CNS border compartments such as the meninges in male rather than female transgenic mice at steady state. Furthermore, distinct hematological profiles as well as enhanced CSF outflow dynamics were observed in mice with endothelial Angpt-2 overexpression, particularly in males. Our findings challenge the current understanding of Angpt-2 as a merely inflammatory cytokine and suggest a role in sex-specific regulation of autoimmune-mediated neuroinflammation.

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P03

GROUP B STREPTOCOCCUS MEDIATED MIMIC OF HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR IMPACTS BLOOD BRAIN BARRIER INTEGRITY

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Bacterial meningitis is a severe infection of the central nervous system (CNS) that occurs when the opportunistic pathogen Group B Streptococcus (GBS) is able to cross the highly selective blood brain barrier (BBB). The BBB is comprised of specialized brain endothelial cells (BECs) that contribute to the barrier function. The mechanisms in which GBS invades the CNS are not completely known. Vascular endothelial growth factor (VEGF) is a secreted angiogenic mitogen that is known to increase vascular permeability and induce BBB dysfunction. RNA sequencing data revealed that VEGF was upregulated during GBS infection in BECs. We investigated the role of VEGF in the infection of GBS through our induced pluripotent stem cell-derived brain-like endothelial cell model, using qPCR techniques where we saw an increase of VEGF expression during infection. We then performed a western blot and demonstrated that VEGF secretion is increased during GBS infection in multiple BEC cell lines further confirming our RNAseq data. A western blot of only GBS media showed binding to a human anti-VEGFA antibody leading to the hypothesis that GBS makes a VEGF mimic impacting the BBB. Further assays revealed that a GBS-secreted factor increased VEGF expression in BECs. To elucidate the role of VEGF in BBB disruption, BECs were treated with recombinant VEGF (rVEGF). TEER was measured over the period of 14 days and decreased significantly at days 5 and 7. Immunostaining was then performed on BECs treated with rVEGF to visualize tight junction disruption. We further demonstrate that VEGF alone is sufficient to disrupt tight junctions and decrease key BBB properties. Overall, our data demonstrates a GBS secreted VEGF mimic acts as a significant mechanism that contributes to barrier dysfunction.

P04

TRANSCRIPTOME ANALYSIS OF THE ASTROCYTES INFECTED WITH THE SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2

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The corona virus disease (Covid-19) pandemic hit the world in 2019 and lasted until 2022. It was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although SARS-CoV-2 is mainly a respiratory pathogen, it was later shown that it has neurotropic and neuroinvasive capabilities and causes serious problems that lead to long-term consequences. In our study, we studied on cell signaling events that occur in astrocytes, the integral part of blood brain barrier, upon infection by SARS-CoV-2. The primary astrocytes were infected with SARS-CoV-2 strain Slovakia/SK-BMC-BA42/2022- variant Omicron VOC BA.5.2. at MOI of 0.2 for one hour. After one hour medium was changed and incubation was continued next 5 hours. The cells were lysed, RNA was isolated and RNA seq libraries (QuantSeq 3' mRNA-Seq) were sequenced on Illumina NextSeq (single-end 75 bp, 13 million reads/sample). RNA-seq results were validated with qRT-PCR. Results revealed that total 91 protein coding genes were significantly upregulated (logFC >1.0) while 53 were significantly downregulated (<-1.0). Pathway enrichment analysis performed by Reactome server revealed that genes involved in several pathways were evoked mainly: signal transduction, innate immunity (including cytokine signaling), cell responses to stress, etc. However, more importantly, several non-coding genes like antisense RNA, lincRNAs, miscRNA, snoRNAs, processed or unprocessed pseudogenes, new transcripts to be confirmed experimentally (TEC) and sense intronic transcripts were significantly evoked. Details transcriptomic analysis will help us gain insight into the molecular processes that occur in SARS-CoV-2 infected astrocytes. Research was funded from APVV-22-0084, VEGA1/0381/23, VEGA1/0348/22, EURONANOMED2021-105.

P05

A HUMAN TISSUE *IN VITRO* MODEL OF THE BLOOD-BRAIN BARRIER IN NEUROMYELITIS OPTICA SPECTRUM DISORDER

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Background: Blood-brain barrier (BBB) breakdown enables auto-antibodies (-Abs) in many central nervous system (CNS) autoimmune diseases to reach their targets, causing inflammatory tissue damage and neurological deficits. One of them is Neuromyelitis Optica Spectrum Disorder (NMOSD), where auto-Abs target the water channel aquaporin-4 (AQP-4) on astrocytes and lead to a complement system-driven immune response.

Research is ongoing to understand how these auto-Abs and effector molecules like complement factors cross the BBB. Human tissue culture BBB models could help bridge the gap in translating animal models to humans. We are currently working on an in vitro model in our lab to resemble NMOSD.

Methods: Our co-culture BBB model consists of a transwell insert, in which we seed immortalized microvascular endothelial cells (HBEC-5i hTERT) and let them grow until monolayer and tight junction formation. The lower compartment incorporates astrocytoma cells (U-87 MG) overexpressing AQP-4. We measure the barrier permeability with 10 kDa dextran. By adding human complement with or without anti-AQP-4 Ab we evaluate cell damage.

Findings: We have characterized the utilized cell types regarding cell-specific markers. Further, we established a protocol to mimic cell damage by human complement after anti- AQP-4 Ab binding.

Outlook: We plan to test various inflammatory conditions by adding different pro-inflammatory cytokines and immune regulators in order to understand BBB- and CNS tissue damage, and auto-Ab infiltration. These insights could aid in developing of new therapies for autoimmune and neurological diseases by enabling the transfer of therapeutic Abs across endothelial barriers.

P06

IMPACT OF ENDOTHELIN RECEPTOR ANTAGONISTS ON PERICYTE FUNCTION

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Pericytes enhance tight junction function through cross-talk with cerebral vascular endothelial cells. However, the dysfunction can lead to microcirculatory disturbances. Endothelin 1 is a key factor in pericyte contraction. We hypothesized that endothelin receptor antagonists could enhance cross-talk by inhibiting endothelin 1-induced pericyte contraction.

This study examined the effects of endothelin receptor antagonists on pericytes and tight junction function using a blood-brain barrier (BBB) model derived from rat primary endothelial cells and pericytes. We administered the dual receptor antagonist bosentan and the selective endothelin A receptor antagonist clazosentan to the BBB model, measured transendothelial electrical resistance (TEER), and evaluated tight junction function through permeability tests.

In the monolayer model, the bosentan group showed a significant TEER increase, while the clazosentan group did not show a notable change. In the coculture model (endothelial monolayer cocultured with pericytes), the bosentan group had a greater TEER increase compared to the control group, surpassing the effect observed in the monolayer model. The Clazosentan group again showed no significant TEER increase, reflecting the monolayer model. Permeability tests corroborated these findings. Western blotting revealed decreased level of endothelin A and B receptor proteins in pericytes.

Compared to the monolayer model, bosentan's protective effect on the BBB was more pronounced in the coculture model, suggesting it enhances pericyte communication. Endothelin receptor antagonists seem to alter receptor expression on pericytes, affecting tight junction function. The lack of effect in the clazosentan group indicates endothelin B receptors on pericytes may be crucial for BBB protection.

P07

EXAMINATION OF MAGNETIC NANOCARRIERS FOR TARGETED DELIVERY ACROSS THE BLOOD-BRAIN BARRIER USING A HUMAN CELL CULTURE MODEL

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The blood-brain barrier (BBB) is essential for protecting the brain, but poses a significant challenge for the delivery of therapeutic agents to treat neurological disorders. By using external magnetic fields, magnetic nanoparticles (MNPs) with BBB targeting ligands can be guided precisely to target sites allowing for drug delivery to the brain. Our aim was to investigate the ability of untargeted and targeted MNPs to cross a human BBB culture model with the use of external magnet.

Fluorescent, starch-coated MNPs were targeted with glutathion through biotin-neutravidin binding. Cell viability was assessed by impedance measurement, MTT assay, and double cell nuclei staining. BBB functions were assessed by measuring transendothelial electrical resistance and permeability of both MNPs and marker molecules was evaluated. Confocal microscopy was used to visualize MNPs and tight junction proteins. Magnetic field was not-toxic to endothelial cells up to 100 mT and MNPs did not damage cells up to a concentration of 300 μg/ml. Only 2% of MNPs were successfully passed across the BBB while the integrity of the barrier was maintained. Both non-targeted and targeted MNPs penetrated into endothelial cells. The non-targeted MNPs consistently resided in almost all cells, whereas the targeted MNPs only entered a subpopulation of cells. The presence of magnetic field did not affect the localization of MNPs and neither the magnetic field nor the MNPs affected the viability of endothelial cells. The penetration of MNPs through the BBB was significantly limited, and the uptake/distribution of non-targeted and targeted MNPs in endothelial cells showed different patterns.

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P08

CAPILLARY PERICYTES REGULATE VASCULAR TONE AND LOCAL BLOOD FLOW IN INFLAMMATION

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Pericytes are the only contractile cells in cerebral capillaries. However, their role in the regulation of capillary diameter, microvascular tone and local cerebral blood flow is far from being completely understood. Furthermore, a large number of CNS disorders is accompanied by inflammatory processes. Therefore, in our present study, we investigated the role of pericytes in the maintenance of capillary tone and how inflammatory mediators could regulate pericyte contractility.

Using primary human pericytes in an in vitro collagen contraction assay, we could demonstrate that TNF-alpha, IL-6 and CCL2 induce a significant pericyte contraction. In order to prove that inflammatory mediators have similar effects in vivo, we used two photon microscopy in mice with labelled pericytes. Inflammatory mediators were administered in the vicinity of identified pericytes using microinjection techniques under continuous monitoring. TNF-alpha induced a slow but significant reduction in the capillary diameter. In addition, using line scan technology, we could show a decrease in red blood cell velocity and a reduction in the number of red blood cells passing the capillary segment in the neighbourhood of the injection. Furthermore, careful ablation of pericytes using the two-photon laser led to a late onset (after 24 hours) dilation of the capillary segment belonging to the ablated pericyte.

Our results indicate that pericytes may have an important role in the maintenance of the capillary tone, and may regulate capillary flow under inflammatory conditions.

P09

PERICYTES MEDIATE NEUROVASCULAR REMODELING IN CHRONIC ARTERIAL HYPERTENSION

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Chronic arterial hypertension restructures the vascular architecture of the brain, leading to a series of pathological responses that culminate in cerebral small vessel disease. Pericytes respond dynamically to vascular challenges; however, how they manifest under the continuous strain of hypertension has not been elucidated. Therefore, in this study, we characterized pericyte behavior alongside hypertensive states in the spontaneously hypertensive stroke-prone rat (SHRSP) model, emphasizing their phenotypic and metabolic transformation. Our results reveal an early transition in PDGFRß+ pericytes toward increased NG2 and CD13 co-expressing subtypes, signaling enhanced pericyte reactivity in an effort to stabilize vascular structures and an inflammatory engagement within the vascular niche in response to hypertensive stress. Gene expression profiling of microvessels revealed altered expression within crucial pathways i.e., angiogenesis, blood-brain barrier integrity, hypoxia and inflammation. Furthermore, we detected that circulating extracellular vesicles from SHRSP alter pericyte mitochondrial membrane potential, highlighting their ability to transmit pathogenic signals that exacerbate vascular remodeling. Detailed metabolic analysis revealed a significant shift toward glycolytic metabolism in pericytes already in initial hypertension, alongside a dysregulation of ATP production pathways. These findings emphasize the transformative influence of hypertension on cerebral pericytes and the extensive consequences on cerebral vascular health.

P10

LONG-LASTING EFFECTS OF FETAL GROWTH RESTRICTION COMBINED TO EARLY POSTNATAL CHRONIC INFLAMMATION ON SELECTED PROPERTIES OF BLOOD BRAIN INTERFACES

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Brain maturation occurs in a defined fluid environment protected by blood-brain interfaces (BBIs). Pre- and perinatal stress conditions such as fetal growth restriction (FGR) or infections are risk factors for neurodevelopmental and later life neurological diseases. We evidenced an early postnatal window of susceptibility to neutrophils infiltration into cerebrospinal fluid (CSF) across the choroid plexuses following systemic exposure to PAM3CSK4, a bacterial TLR2 agonist. At postnatal day 3 (P3), this pleocytosis is more important in some FGR than in control animals.

In this study, we explore both the short and long-term effects of FGR, combined or not to chronic postnatal systemic inflammation, on the integrity of BBIs and their responsiveness to later-life systemic infection.

We use a clinically relevant rat model of FGR induced by a gestational low protein diet. Chronic inflammation is induced by daily injection of PAM3CSK4 from P2 to P5. Kin CSF and Kinapp brain for [14] C-sucrose, used as indices of BBI integrity, are higher at birth (P3), but not later on (P30), in FGR as compared to control animals. Several FGR animals subjected to early postnatal chronic inflammation responded to an additional PAM3CSK4 exposure at P30 with significant pleocytosis and altered BBI integrity in selected brain areas.

Overall, the data show that BBIs are relatively resilient to FGR condition, as the alteration of their integrity is transient. However, FGR coupled to early postnatal chronic inflammation, reduces the BBI ability to cope with later-life inflammatory stresses.

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P11

PROGESTERONE TREATMENT RESTORES BLOOD-BRAIN BARRIER FUNCTION AND IMPROVES LONG-TERM MEMORY AND LEARNING IN A RAT MODEL OF CHRONIC CEREBRAL HYPOPERFUSION

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Cerebral blood flow supplies oxygen and nutrients to support normal brain function. Aging is associated with chronic cerebral hypoperfusion (CCH). These blood flow age-related alterations might be attenuated by progesterone (P4), a neurosteroid which has been proven to exert pleiotropic neuroprotective effects in several models of brain injury. The aim of this study was to evaluate the effects of P4 on the blood-brain barrier (BBB) in the short term and on spatial learning and memory in the long term in rats subjected to CCH. Male Sprague-Dawley rats (12-14 months old) were randomly distributed in the following groups: CCH+vehicle; CCH+P4 (8 mg/kg/day) and sham procedure as a control. At seven and fourteen days after CCH, the function of the BBB was evaluated through permeability assays by systemic administration of a cocktail containing Evans blue and Na-fluorescein tracers. In addition, the expression of BBB tight junction proteins and inflammation factors was evaluated by western blot. At 180 days later, memory and learning were evaluated using the Barnes maze and the novel object recognition test. CCH induced BBB dysfunction, decreased tight junction protein expression, increased inflammatiory factors and induced an impairment in memory and learning. Treatment with P4 improved the BBB function, restored the expression of the tight junction proteins, decreased neuroinflammation, and preserveds learning and memory. These results suggest that, in old male rats with disrupted blow-flow, P4 plays an important role in restoring BBB function, which may contribute to the neuroprotective effects that have been previously reported.

P12

AMYLOID-BETA IMMUNOTHERAPY IN ALZHEIMER'S DISEASE: AN IN VITRO STUDY DEPICTING THE MOLECULAR MECHANISMS

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Alzheimer's disease (AD) is the most common form of dementia, with the main pathological hallmark being the accumulation of amyloid-β (Aβ). Treatment options are exceedingly limited, and until now there have been no therapies that selectively target amyloid plaques with the potential to effectively attenuate disease progression. Aducanumab has been a breakthrough, being the first immunotherapy approved for AD, providing a glimmer of hope for people worldwide. Despite this, the comprehensive molecular mechanisms are still not clearly defined and there is very limited in vitro data describing the molecular and cellular mechanism of action. Our aim was to test the treatment in a blood-brain-barrier (BBB) co-culture model, examining the mechanisms of clearance by microglial cells in response to the drug. Using fluorescence activated cell sorting, combined with confocal microscopy and paracellular permeability, we identified that Aducanumab increased the rate of Aβ engulfment by microglial cells, without affecting BBB integrity. We observed that microglial cells become phagocytically activated in response to the presence of Aβ in the monomeric and especially the oligomeric form, with a clear release of pro-inflammatory cytokines (e.g. TNF-α) overturned by the treatment with Aducanumab at increasing Aβ concentrations. Aβ phagocytosis was further increased in the presence of Aducanumab even when applied via the endothelial layer with no effect on the endothelium tightness. These data suggest that Aducanumab is not toxic to the endothelial cells and contributes to the monomeric or oligomeric Aβ clearance through a pro-resolving mechanism at higher plaque densities.

P13

EXPRESSION OF ALPHA SMOOTH MUSCLE ACTIN DECREASES WITH AGEING AND INCREASES UPON LUMEN OBSTRUCTION IN MOUSE BRAIN PERICYTES

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Cerebral pericytes are mural cells covering brain microvessels, organized as ensheathing, mesh and thin-strand pericytes. These latter two, together called capillary pericytes, have low levels of alpha smooth muscle actin (α-SMA), regulating basal vascular tone and applying a slow influence on cerebral blood flow.

Pericytes are subject to alterations in ageing which may be even more pronounced in pathologies, including microinfarcts, which usually affect a large number of vessels in the ageing brain. We modelled this condition by injecting 10 μm-size microspheres into the circulation of mice resulting in the occlusion of capillaries covered by ensheathing and mesh pericytes.

We observed that α-SMA and Acta2, the gene encoding it, as well as TGF-β1/*Tgfb1,* the major regulator of α-SMA, decreased during ageing in cerebral microvessels. In the vicinity of the microspheres stalled in the capillaries, expression of α-SMA increased significantly in both ensheathing and especially in mesh pericytes, both in young (2 to 3 months of age) and old (24 months of age) mice. On the other hand, γ-actin was detected in endothelial cells, but not in pericytes, and decreased in microvessels of microsphere-containing hemispheres.

Altogether, our data show that obstruction of cerebral microvessels increases α-SMA expression in pericytes in both age groups, but this does not compensate for the lower expression of the contractile protein in old animals. Increased α-SMA expression may lead to constriction of the obstructed vessels probably aggravating flow heterogeneity in the aged brain.

P14

DOUBLE-STRANDED DNA SENSING CGAS-STING SIGNALING PATHWAY AND NEUROINFLAMMATION IN A RAT CO-CULTURE MODEL OF THE BLOOD-BRAIN BARRIER

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Double-stranded DNA coming from viruses, bacteria, eukaryotic parasites, cancerous or apoptotic cells is recognized by the cGAS-STING signaling pathway. The pathway activates transcription of interferon stimulated genes and proinflammatory cytokines. The blood-brain barrier (BBB) as an immunological actor can play an important role in brain diseases, such as Parkinson's disease, which is characterized by α-synuclein oligomer aggregates. In this BBB-affecting neurodegeneration, mitochondrial DNA can be released into the cytosol and activate the cGAS-STING pathway. Our aim was to study the cGAS-STING pathway in the different cell types and a rat co-culture model of the BBB before and after treatment with α-synuclein monomers (αSM) and oligomers (αSO). Activation of the cGAS-STING pathway did not change transendothelial electrical resistance (TEER) or barrier permeability, but it induced the transcription of the interferon stimulated gene Viperin and the proinflammatory cytokine tumor necrosis factor-α (TNFα) in rat brain endothelial cells (BECs). The treatment with αSO, but not αSM, decreased TEER values and induced the transcription of Viperin and TNFα in BECs. In rat astrocytes, αSO treatment increased not only Viperin and TNFα mRNA levels, but also other proinflammatory cytokines, such as interleukin-1β and interleukin-6. In conclusion, cGAS-STING pathway can be activated in the rat co-culture model of the BBB, this activation induces immune signaling pathways in BECs, but it does not influence barrier integrity. The αSO disrupts integrity of the BBB, and it activates the cGAS-STING pathway in BECs and astrocytes supporting the idea of using cGAS-STING as a therapeutic target in neuroinflammation.

P15

UNDERSTANDING ACTIVATION OF THE UNFOLDED PROTEIN RESPONSE AT THE BLOOD-BRAIN BARRIER DURING GROUP B STREPTOCOCCAL INFECTION

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Bacterial meningitis is a life-threatening infection of the central nervous system (CNS) that occurs when blood-borne bacterial pathogens disrupt the brain endothelial cells (BECs) of the blood-brain barrier (BBB) and enter the CNS. Group B Streptococcus (GBS) is the leading cause of neonatal meningitis and mechanisms of how the BBB fails to protect the CNS during infection remain unclear. The endoplasmic reticulum (ER) is a subcellular compartment that aides in protein folding and secretion. When misfolded proteins accumulate, the ER activates the unfolded protein response (UPR) to increase the folding capacity of the cell. Activation of the UPR is implicated in many disease states, including some bacterial infections. Examination of our RNAseq analysis of BECs subject to GBS infection suggest that the ATF6 branch of the UPR is activated during GBS infection. We have found that GBS induces ER stress that results in the upregulation of the protein chaperone GRP78 as well as inflammatory cytokines and chemokines. This suggests that ER stress contributes to activation of BECs, and treatment of BECs with chemical ER-stressors tunicamycin and thapsigargin also recapitulates upregulation of GRP78 and select inflammatory markers. Interestingly, chemical induction of ER stress also results in the upregulation of tight junction proteins Occludin, and ZO-1, indicating that ER stress may act as a protective response by the BBB against GBS meningitis. Taken together, our findings suggest that the UPR is activated in BECs during GBS infection, while the role of this response remains under investigation.

P16

N,N-DIMETHYLTRIPTAMINE REDUCES CEREBRAL ISCHEMIA-INDUCED INFLAMMATION OF THE NEUROVASCULAR UNIT

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N,N-dimethyltriptamine (DMT) is the psychoactive compound of the ayahuasca brew originally used in indigenous cultures. It is an endogenous ligand of sigma-1 receptors and has been shown to reduce neuroinflammation. In the present study we aimed to examine DMT effects on the neurovascular unit following cerebral ischemia-reperfusion injury in rats. Ischemia was induced by middle cerebral occlusion for 60 min. During reperfusion, three experimental groups were formed: controls received an intra-peritoneal (ip) bolus of vehicle, DMT treated animals received an ip bolus of 1 mg/kg body weight (bw) DMT dissolved in vehicle followed by 2 mg/kg bw of DMT via osmotic pump for 24h, and the third group received 1 mg/kg bw + 2 mg/kg bw of BD1063, a sigma1 receptor antagonist, together with the DMT. The brains were processed for immunohistochemical analysis to detect the expression of claudin-5 and aquaporin-4 (AQP4) as blood-brain barrier (BBB) markers, and the expression of GFAP and Iba-1 as markers of glial components of the neurovascular unit. In controls, the fluorescence intensity of claudin-5 decreased and that of AQP4 increased on the injured side. These changes were attenuated by DMT. The fluorescence intensity of GFAP showed no change, while that of the microglia marker Iba-1 was increased in every injured group. The injury induced a strong reactive microgliosis in controls. In the DMT group, the morphology of reactive microglia suggested a decreased inflammation. Most DMT effects were blocked by BD1063. Thus, DMT may effectively restore BBB function following ischemia-reperfusion acting via sigma1 receptors.

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P17

HUMAN IMMORTALIZED CELL-BASED BLOOD-BRAIN BARRIER MODELS ARE A USEFUL TOOL FOR EVALUATION OF BRAIN PERMEABILITY OF AAV VECTORS

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[Purpose] Adeno-associated virus (AAV) is a promising gene delivery vector to the human brain. Development of more effective and safer gene therapy for brain diseases requires improvement of blood-brain barrier (BBB) permeability of AAV vectors, in which in vitro human BBB models are expected to play a pivotal role as shown in the previous report (Merkel et al., J Neurochem, 2017;140:216-230). Therefore, in this study, we aimed to clarify whether human immortalized cell-based transwell BBB models (hiBBB) can evaluate the brain permeability of AAV vectors.

[Methods] After constructing the hiBBB models, the permeability of AAV9 and AAV2, both carrying the GFP gene, was evaluated by quantifying the AAV genomes found in the brain compartment. The GFP expression in the brain microvascular endothelial cells (BMEC) was examined by fluorescence microscopy.

[Results and Discussion] The results of the permeability assays showed that AAV9 exhibited higher BBB permeability levels than those of AAV2 (c.a. 6-fold). The fluorescence microscopic analyses of the BMEC showed higher GFP expression levels in the AAV2-exposed BMEC than those to AAV9, indicating that AAV2 can more readily enter the gene expression pathway in BMEC.

[Conclusions] The hiBBB models clearly distinguish the differential BBB permeability of AAV9 than that of AAV2, which are in consistent with the results obtained from human primary cell-based BBB models (Merkel et al.). Therefore, it can be expected that the hiBBB models are also useful for exploring an AAV vector with excellent BBB permeability.

P18

IN VIVO DELIVERY OF BBB-PERMEABLE CYCLIC PEPTIDE-FUSED MONOCLONAL ANTIBODIES TO THE MOUSE BRAIN

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Monoclonal antibodies (mAbs) exhibit potential to address the unmet medical needs of patients with central nervous system (CNS) disorders. However, effective mAb delivery to the brain remains a challenge. Therefore, adaptable techniques for mAb delivery across the blood–brain barrier (BBB) are urgently needed to treat CNS disorders. We previously identified a BBB-permeable cyclic peptide (SLS) that facilitates the delivery of macromolecules across the BBB to the brain. In this study, we investigated the in vivo brain delivery efficiency of SLS peptide-fused mAbs across the BBB. The SLS peptide was fused to the C-terminus of the trastuzumab heavy chain (TCH; a model mAb) using a (G4S) 3 linker (TCH-H-(GS)3-SLS). In vivo studies in mice showed a 5.7-fold increase in the brain levels of TCH-H-(GS)3-SLS compared to those of TCH one hour after intravenous injection (2 mg/kg), with no significant differences in serum concentrations. Immunohistochemistry confirmed the distribution of TCH-H-(GS)3-SLS in the brain parenchyma. Notably, peptide fusion did not significantly alter the levels of TCH-H-(GS)3-SLS compared to those of TCH in isolated brain capillaries, indicating that fusion of the SLS peptide increased mAb delivery to the brain. In conclusion, our findings suggest fusion of the SLS peptide as an effective strategy for mAb delivery across the BBB to the brain. Moreover, our method is theoretically applicable to all mAbs, thus facilitating the development of novel mAb-based therapeutics for CNS disorders.

P19

BRAIN PERICYTES ENHANCE THE EXPRESSION AND PLASMA MEMBRANE LOCALIZATION OF MFSD2A IN BRAIN ENDOTHELIAL CELLS THROUGH PDGF-BB/PDGFRB SIGNALING PATHWAY

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Brain actively takes up nutrients through various transporters on brain microvessel endothelial cells (BMECs). In particular, docosahexaenoic acid (DHA) is the most abundant nutrient in the brain and is important for many neurophysiological functions. A major transporter for DHA at the Blood-brain barrier (BBB) is the major facilitator superfamily domain-containing protein 2a (MFSD2A), which is expressed exclusively in BMECs. Although brain pericytes regulate the expression of MFSD2A on BMECs, its mechanism remains unclear. Here, we used non-contact coculture BBB models consisting of primary cultures of rat brain endothelial cells (ECs) and rat brain pericytes (PCs). In addition, to determine whether PDGF-BB/PDGFRβ signaling between ECs and PCs affects the expression and plasma membrane localization of MFSD2A protein in ECs, we examined the impact of AG1296 (the inhibitor of PDGF receptor) and Pdgfrb knockdown PCs on non-contact coculture models. After 3 days of coculture, expression levels of MFSD2A were evaluated by western blot analysis and immunofluorescent staining. The impact of PCs on brain endothelial DHA uptake was assessed by [14 C] DHA uptake by ECs cocultured with PCs. In ECs cocultured with PCs, MFSD2A protein expression and plasma membrane localization were significantly increased compared with ECs monolayer. The increases of protein expression and membrane localization of MFSD2A were inhibited by AG1296 and Pdgfrb knockdown PCs. Furthermore, PCs significantly increased [14 C]DHA uptake by ECs. Our findings suggested that PCs enhanced the MFSD2A protein expression and plasma membrane localization in ECs by PDGF-BB/PDGFRβ signaling between PCs and ECs.

P20

MAPK SIGNALING AT THE BLOOD BRAIN BARRIER DURING COXSACKIEVIRUS B3 INFECTION

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The Blood Brain Barrier (BBB) is a highly specialized cellular barrier composed of brain endothelial cells (BECs) that prevent the passage of toxins and pathogens into the Central Nervous System (CNS). Coxsackievirus B3 (CVB3) is a non-polio enterovirus that causes viral myocarditis, pancreatitis, and is a leading cause of viral meningitis. The mechanism through which CVB3 penetrates the BBB is unclear, which may, in part, be due to the lack of robust BBB modeling systems. Our lab models the BBB using induced-pluripotent stem cell derived brain-like endothelial cells (iBECs), as they recapitulate important barrier properties of the human BBB, such as tight junctions and high transendothelial electrical resistance (TEER). We have demonstrated that CVB3 infects iBECs and elicits a significant change in gene expression at five days post infection (PI), including upregulation of antiviral and interferon genes as well as enrichment of MAPK signaling. To further study this, we inhibited the MEK/ERK pathway with the ERK1/2 inhibitor U0126, and observed an increase in Green Fluorescent Protein (GFP) and Viral Capsid (VP1) protein abundance in treated CVB3 infected cells at five days PI. After treatment with U0126, there is a decrease in the abundance of antiviral proteins IFITM1, IRF7, and IFITM2/3 in infected cells. We hypothesize that the MEK/ERK signaling pathway is vital for the antiviral response against CVB3 infection in the BBB. In the future, we aim to observe inhibition of other stages in the MEK/ERK pathway and other branches of MAPK signaling.

P21

THE TRYPTOPHAN-KYNURENINE PATHWAY- THERAPEUTIC STRATEGY FOR NEUROPROTECTION IN TAUOPATHIES

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that deteriorates cognitive functions, characterized by the intracellular accumulation of abnormal filaments of the microtubule-associated protein tau, amyloid-β plagues (Aβ), and neuronal apoptosis in the central nervous system (CNS) releasing pro-inflammatory cytokines with hyper-reactive brain immune cells (microglia and astrocytes). Moreover, these deposits are associated with systemic inflammation and disruption of the many signaling pathways including the kynurenine pathway. The tryptophan-kynurenine pathway (TKP) is functionally maintained by the catabolism of the essential amino acid, L-tryptophan. This pathway generates various metabolites specifically the kynurenic acid (KA), a neuroprotective metabolite in the CNS. In neuroinflammatory conditions, under the influence of inflammatory cytokines (INF-Y) and reactive oxygen species (ROS), a key regulatory enzyme indoleamine 2,3-dioxygenase (IDO) is induced, declining the neuroprotective metabolite levels and increasing the production of a neurotoxic agent such as Quinolinic acid (QA). The IDO-induced QA over-stimulates the excitatory receptor N-methyl-D-aspartate (NMDA) acting as an agonist whereas, KA acts as an antagonist of this receptor modulating the functioning of the brain. We aim to screen analogs of kynurenic acid having similar biological activity, to modulate the neuroinflammation in the CNS and its functions using an in-vitro blood-brain barrier (BBB) model as well as an in-vivo model using the transgenic rats. Our screening of various analogs identified few compounds with both high blood-brain barrier permeability and promising biological activities. These findings suggest potential for effective treatment of neuroinflammatory conditions.

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P22

P-GLYCOPROTEIN DISRUPTION IN BLOOD-BRAIN BARRIER HOST CELLS AS A RESULT OF STREPTOCOCCUS AGALACTIAE VIRULENCE FACTOR

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The blood-brain barrier (BBB) is a highly specialized network comprised of brain endothelialcells (BECs) that facilitates the passage of molecules to and from the central nervous system. The barrier is characterized by the presence of tight junctions, reduced endocytosis rates, and increased expression of a variety of highly specific transport proteins. P-glycoprotein (P-gp) is one such efflux transporter that is highly expressed in the BBB and plays a critical role in preventing the entry of foreign substances into the brain. Streptococcus agalactiae, the leading cause of neonatal meningitis, is a Gram-positive pathobiont that we have demonstrated disrupts P-gp function through unknown mechanisms. We hypothesize that the S. agalactiae β-hemolytic toxin is responsible for P-gp disruption at the BBB through both direct effects and activation of a host cell signaling pathway. The human BBB can be modeled in vitro using an induced pluripotent stem cell-derived model, differentiated to mimic BECs (iBECs). The infection of iBECS with various S. agalactiae strains, including a hyperhemolytic mutant and a mutant unable to produce toxin, reveals altered P-gp function, measured using an established fluorescent P-gp substrate accumulation assay. Additionally, we are treating iBECs with inhibitors of suspected interacting pathways with the goal of promoting a rescue effect of P-gp function, expression, and abundance during infection. The intersection of these two methods provides a platform for the discovery of the responsible S. agalactiae gene and subsequent mechanism of BBB disruption, resulting in more favorable treatment options.

P23

CHARACTERIZATION OF A NEW HUMAN CO-CULTURE MODEL OF ENDOTHELIAL CELLS, PERICYTES AND BRAIN ORGANOIDS IN A MICROFLUIDIC DEVICE

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The blood-brain barrier (BBB) protects the brain and provides oxygen and nutrients for the central nervous system (CNS), but it also restricts the entry of pharmaceutical drugs into the brain. Cell culture models are essential to investigate cerebral drug delivery. Microfluidic chip devices allow complex and physiological modelling of the BBB. Induced pluripotent stem cell (iPSC) based technologies, the formation and use of human brain organoids provide simplified 3D modeling. Our aim was to (1) create and optimize a new, dynamic cell culture lab-on-a-chip model by the co-culture of a BBB model and human midbrain organoids, and to (2) examine BBB properties and functionality in the presence of organoids. Human stem cell derived endothelial cells and brain pericytes were co-cultured to establish the BBB model (Cecchelli et al., 2014). Human midbrain organoids were differentiated from iPSCs from healthy people and Parkinson's disease patients (Nickels et al., 2020). The barrier integrity of the BBB model was investigated in the presence of midbrain organoids in a dynamic setup by the measurement of impedance and permeability for fluorescent markers. The morphology of brain endothelial cells was examined by immunostaining for tight junction proteins. Functionality of the model was tested by the passage of targeted nanoparticles across the BBB and by characterizing the uptake into the organoids. We found appropriate BBB maturation and integrity in the presence of brain organoids. Nanoparticles crossed and entered the organoids effectively. This complex organ-on-a-chip system can be a valuable tool for further experiments in drug testing. The project was supported by the NKFIH, NNE-129617 & OTKA-K 143766 (to M.A.D.),
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P24

INFLAMMATORY CHANGES OF CHOROID PLEXUS IN TAUOPATHIES

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In our study, we examined how the blood-cerebrospinal fluid barrier (BCSFB) facilitates communication between the peripheral and central nervous systems (CNS) in the context of tauopathy. While the exact mechanisms behind disrupted BCSFB function in Alzheimer's disease (AD) are still not fully understood, it is believed to be linked to epithelial cell activation and cytokine-mediated inflammation. Our research findings suggest that changes in BCSFB function, which result in altered permeability and transport, are associated with the immune cells and the expression of specific inflammatory proteins. This study adds to the growing evidence that inflammation may contribute to the early stages of AD by affecting the pathogenesis of altered BCSFB function.

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P25

A BLOOD-BRAIN BARRIER MODEL FOR LIPOSOMAL DRUG TARGETING - CHARACTERISATION OF HIPSC-DERIVED ENDOTHELIAL CELLS FOR IN VITRO MODELLING OF THE BBB

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Blood-brain barrier (BBB) models represent a valuable tool in assessing barrier characteristics as well as drug permeability into the brain. Human induced pluripotent stem cells (hiPSCs) can be differentiated to acquire BBB properties and can therefore play a pivotal role in the development of therapeutic options for CNS diseases. Here, hiPSCs were cultivated on Matrigel and subsequently differentiated to brain microvascular endothelial cells (BMECs) on collagen IV/fibronectin coating 1 . After confirming the formation of a tight cell monolayer via transendothelial electrical resistance (TEER) measurement (TEER value ≥ 1000 Ω·cm 2), potential targets for drug delivery, namely LRP1, Mfsd2a and TfR were identified on mRNA and protein level. Mfsd2a and TfR served as targets for antibody decorated liposomes in uptake experiments. Uptake was significantly higher compared to plain liposomes, thus confirming that hiPSCs can be used as a characterized platform for controlled release formulations and drug transport across the blood- brain barrier.

P26

ALTERED EXPRESSION OF PATTERN RECOGNITION RECEPTORS IN HUMAN ASTROCYTES CAUSED BY NEUROINVASIVE BACTERIA

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Astrocytes, the most abundant glial cells in the brain, play a variety of important roles in the CNS, including initiating immune responses during infections. The goal of this study was to evaluate astrocytes' response to neuroinvasive pathogens in vitro using transcriptomic analysis. Primary human astrocytes were incubated separately with six bacteria (Borrelia bavariensis, Escherichia coli K1, Klebsiella pneumoniae, Listeria monocytogenes, Neisseria meningitidis, and Streptococcus pneumoniae) for 6 hours at 37°C and 5% CO 2 . The RNA was then isolated, libraries for RNA sequencing were prepared (QuantSeq 3' mRNA-Seq) and sequenced on Illumina NextSeq, single-end 75 bp, to a minimal depth 13 million reads per sample. RNA-seq results were validated with qRT-PCR on randomly selected genes. Pathway enrichment analysis was performed using Reactome server of EMBL. The results revealed that pattern recognition receptor (PRR) genes such as TLR2, TLR3, and NOD2 were significantly overexpressed. Surprisingly, TLR4 and TLR5, which recognize bacterial lipopolysaccharide and flagella, were not evoked. Activation of PRRs can activate RIP2 kinase, NF-κB, caspase-1, and MAP kinases, which regulate inflammation. RIP2 and NFKB1/2 were only upregulated during E. coli infection, whereas interleukin-1 receptor associated kinase 3 (IRAK3), which is normally expressed in peripheral blood cells, was upregulated in nearly all bacterial infections. Our findings may contribute to a better understanding of the signaling events and pathways that occur in astrocytes during brain infection caused by various bacteria. Research was funded from APVV-22-0084, VEGA1/0381/23, VEGA1/0348/22, EURONANOMED2021-105.

P27

INVESTIGATION OF BLOOD-BRAIN BARRIER CHANGES IN ACUTE PANCREATITIS: A CELL CULTURE AND CLINICAL STUDY

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Acute pancreatitis (AP) is an inflammatory gastroenterological disease, during which about 4% of all patients develop disturbance in consciousness. Besides this sypmtom among the severe AP cases 10 % show serious neurological involvement manifesting in pancreatic encephalopathy. Earlier research from our laboratory showed blood-brain barrier (BBB) permeability elevation in a rat non-invasive AP model. Now our goal was to identify potential BBB injury in pancreatitis patients. For this study we used serum from mild, moderate and severe AP patients to identify BBB opening by measuring neuron specific enolase (NSE) and S100B presence in the blood. Cultured brain endothelial cells were also treated with 20% human sera. Functional tests: permeability, transendothelial electrical resistance, ROS/NO production were analyzed. Morphological investigations: interendothelial junctions, adhesion molecules, mitochondrial network visualization were done. Surface glycocalyx integrity analysis: lectin staining, zeta potential and streaming potential measurements were performed. We found elevated NSE and S100B levels in the serum of patients with mild, moderate and severe AP. Serum treatment decreased the brain endothelial cell layer resistance and elevated permeability. Key interendothelial junctional and adhesion molecule expression and morphology were affected. ROS production was increased and mitochondrial network was also damaged. Our results show, that treatment with the AP patient sera influences many important BBB properties such as barrier integrity, level of oxidative stress and junctional morphology. The fact that BBB leakage markers were found in the blood of patients from all AP severity groups draws the attention to the serious neurological side effects of the disease.

P28

BONE MARROW-DERIVED CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN STROKE: A PROXY OF NEUROVASCULAR UNIT REPAIR

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Stroke is a global leading cause of disability and death. Ischemic stroke triggers the production and mobilization of Endothelial Progenitor Cells (EPCs) from the bone marrow into the bloodstream. EPCs, a minor population of circulating mononuclear cells with endothelial and stem cell properties, peak at day 7 post-stroke and higher counts associate with improved neurological outcomes. Circulating Endothelial Cells (CECs) serve as specific and sensitive markers of endothelial damage in various conditions, although their clinical application is primarily research-focused.

This study hypothesizes that elevated EPC levels at day 7 post-stroke could predict better functional outcomes and reduced brain injury, assessed via MRI and functional evaluations. By analyzing CECs and EPCs post-stroke, the study aims to clarify their contributions to neurovascular repair. A protocol was developed to quantify CECs and EPCs using multiparametric flow cytometry on peripheral blood mononuclear cells. Blood samples from stroke patients are collected between days five and seven and processed immediately to ensure accurate cell counts.

The specific phenotypes of CECs and EPCs remain under investigation and this study uses specific markers to distinguish between them. Absolute cell counts were determined using a dual-platform counting method.

EPCs are crucial for vascular health appearing as a promising target for stroke treatment through re-endothelization, angiogenesis, and vasculogenesis. Adequate EPC production post-stroke may reduce the risk of future vascular events and this study aims to validate EPC counts as prognostic markers to enhance the prediction of rehabilitation outcomes and improved patient care.

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P29

ANTIDEPRESSANT DRUGS MODIFY THE PERMEABILITY OF A HUMAN BLOOD-BRAIN BARRIER CULTURE MODEL BY ALTERING MEMBRANE TRAFFICKING

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The selective serotonin reuptake inhibitor (SSRIs) class of antidepressant drugs is considered a potent candidate for drug repurposing. It was observed that fluvoxamine, a canonical member of this class, modulated the endocytic membrane trafficking in non-neuronal cell types. However, the mechanisms underlying its mode of action has remained unclear. Our aim was to investigate the effects of fluvoxamine on endocytic pathways of a human culture model of the blood-brain barrier. Real-time impedance measurements indicated no cytotoxic effect of fluvoxamine on stem cell derived human brain endothelial cells (hEC) in a therapeutic range of concentration (30 nM-1 μM). Fluvoxamine at 80 nM concentration increased the internalization of Lucifer yellow, a small hydrophilic dye and a marker of fluid phase endocytosis, but not that of a larger endogenous protein, galectin-1, which is internalized by a receptor-mediated mechanism. We also observed a two-fold higher brain endothelial uptake of Lucifer yellow compared to galectin-1. In the following, the permeability was investigated: fluvoxamine treatment (80 nM) increased the permeability of galectin-1 and the passive paracellular marker molecule 4 kDa FITC-dextran across the co-culture BBB model in 1- and 2-hour time points compared to the control groups. Furthermore, fluvoxamine treatment at 80 and 400 nM concentrations for 1 hour increased the labeling for EEA1 positive early endosomal compartment and LAMP1 positive lysosomes in hEC. In conclusion, we demonstrated that fluvoxamine increases the permeability of a human culture BBB model by modifying the endocytic pathways, which then may be exploited for controlling drug delivery to the brain.

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P30

TOWARD CREATING AN ORTHOGONAL BRAIN GATE: DEVELOPMENT OF SYNTHETIC RECEPTORS FOR THE TRANSPORT OF DRUGS ACROSS THE BLOOD-BRAIN BARRIER

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Pathologies affecting the Central Nervous System, including brain tumors, represent a large and growing health problem for society. The efficiency of current diagnostic and therapeutic molecules is limited due to the presence of the Blood-Brain Barrier (BBB). The BBB is the largest exchange interface between the brain parenchyma and the rest of the body. It acts as a highly selective molecular sieve by tightly controlling the transport of the majority of substances. Several strategies have been developed to improve brain delivery of drugs to a limited extent, either by disrupting the integrity of the BBB or by utilizing natural transport mechanisms. The Orthogonal Brain Gate (OBGate) project, funded by the European Research Council, pursues the development of synthetic transport systems facilitating transcytosis of drugs across the BBB. Here we will present our advances in the development of a transport system based on a natural receptor that harbors a synthetic epitope enabling orthogonal recognition by selective exogenous ligands. With this receptor, we have obtained promising expression and binding results in vitro and we are currently analyzing its transport capacity in different BBB models. The OBGate concept paves the way for new engineered transport systems that will shed light into receptor-mediated transcytosis and potentially enable more efficient delivery of diagnostic and therapeutic molecules to the brain.

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EXPLORING THE INFLUENCE OF TAU PATHOLOGY ON LIPID METABOLISM

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Tauopathies are a group of neurodegenerative disorders marked by cerebral atrophy, hyperphosphorylation, abnormal aggregation of tau filaments into intracellular neurofibrillary tangles, and chronic neuroinflammation. While lipid metabolism dysregulation is a recognized factor in aging and neurodegeneration, its intricacies remain poorly understood. To explore the impact of tau pathology on lipid metabolism, we conducted a comprehensive targeted lipidomic and metabolomic analysis of brain tissue, cerebrospinal fluid (CSF), and plasma from transgenic rats expressing human truncated tau. We measured specific biofluid markers of tau pathology (total-tau and neurofilament-light-chain) along with hyperphosphorylated and aggregated tau forms in brain tissue to assess the effect on metabolic profiles. Our findings reveal significant dysregulation of lipid metabolism in brain tissue, with an even greater impact observed in the CSF due to neurofibrillary pathology. The most notable changes were detected in the subclasses of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidylcholine, and sphingomyelin. However, these lipid alterations in the CSF and brain were not reflected in the plasma. Further analysis indicated that these lipid changes are correlated with tau pathology in brain tissue. Additionally, we discovered that tau pathology induces the formation of lipid droplets both in vitro and in vivo. Our results underscore the critical role of lipid metabolism in tau pathology and highlight its connections with key hallmarks of neurodegenerative diseases, such as neuroinflammation, glial activation, and hyperphosphorylation.

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VISUALIZING AND FUNCTIONAL ASSESSMENT OF IN VITRO COMPLEX BLOOD-BRAIN BARRIER

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BBB models are essential for investigating the transport efficacy of drugs targeting the brain. They shed light on key mechanisms that underpin pathologies involving brain tissue. The goal of this study was to develop a complex in vitro model of the BBB. For this purpose, three cell types - human brain endothelial cells (hBMECs/D3, Sigma Aldrich), pericytes, and astrocytes (ScienCell) were used. Cells were seeded in 12-well inserts with 3.0 µm pore size on PET membrane (CellQuart). Inserts were pre-coated with Collagen type I and poly-L-lysine. Before seeding, intravital tracer dyes were included, to visualize pericytes with DeepRed dye and astrocytes with blue dye (Invitrogen). For hBMECs/D3 no intravital dye was used. After 5th day of seeding all the cell types, TEER values were measured with EVOM. Integrity of the barrier was achieved, when TEER values were higher/or equal to 45 Ω/cm2. Barrier was incubated with dextran-CF770 (negative control) or angiopep 2-Cy5.5 (positive control) added in luminal chamber. After 5 h assay, 3,39% of dextran-CF770 and 22.70% of angiopep-2 Cy5.5 passed through the BBB to abluminal chamber. Microscopic observations were conducted under Cytation7 microscopic multidetector reader (Agilent). hBMECs were observed in apical site of insert using bright field. Pericytes and astrocytes were observed in basolateral site of insert by red and blue filters. Image montage was done on ImageJ software. Constructed BBB model will be used for future *in vitro* assays of therapeutics delivery through the barrier. Research was funded from APVV-22-0084, VEGA1/0381/23, VEGA1/0348/22, EURONANOMED2021-105.

P33

PNEUMOCOCCUS INITIATES PRO-INFLAMMATORY SIGNALING AND SUPPRESSES TIGHT JUNCTION EXPRESSION IN IPSC-DERIVED BRAIN-LIKE ENDOTHELIAL CELLS

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The blood-brain barrier (BBB) refers to the highly specialized network of brain endothelial cells that comprise the microvasculature of the brain. The BBB uses a variety of strategies to maintain brain homeostasis, including the expression of efflux transporters and complex tight junctions, and suppression of inflammatory signaling pathways and cytokine production. During bacterial meningitis, however, key attributes of the BBB become dysregulated, allowing the entry of pathogens to the CNS. Streptococcus pneumoniae is the leading cause of meningitis world-wide, but many of the mechanisms whereby it disrupts the BBB remain unknown. Here, we utilize a novel induced pluripotent stem cell-derived brain-like endothelial cell model (iBECs) to study the interaction between pneumococcus and the BBB. First, we show that pneumococcus promotes the expression of SNAI1, a transcriptional repressor that inhibits tight junction expression. Moreover, we demonstrate that pneumococcal infection decreases the expression and abundance of several tight junction proteins including ZO-1. Additionally, although the BBB is typically immune quiescent, we show that iBECs are activated by pneumococcal infection, as indicated by the induction of various cytokines and chemokines. Surprisingly, we do not find an increase in NF-κB abundance or translocation, suggesting an alternative pathway for immune activation. We also find that pneumococcus promotes the expression of the angiogenic factor VEGF, further indicating a loss of BBB identity and integrity. Taken together, these findings suggest that pneumococcus potentiates aberrant signaling pathways to suppress tight junctions and promote inflammatory mediators at the BBB during the initiation of meningitis.

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ALANINE AND GLUTATHIONE TARGETING OF DOPAMINE- OR IBUPROFEN-COUPLED POLYPEPTIDE NANOCARRIERS ELEVATES CROSSING ACROSS THE BLOOD-BRAIN BARRIER AND PROTECTIVE EFFECTS

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Targeting the blood-brain barrier (BBB) is the key to the effective brain delivery of nanocarriers. We have previously discovered that ligand combinations of BBB nutrient transporters, especially alanine and glutathione, increase the permeability of vesicular and polypeptide nanocarriers across the BBB. Polypeptides are versatile nanoplatforms to combine high functionality with excellent biocompatibility. Our aim here was to investigate whether the alanine and glutathione targeting molecules can also promote the efficient transfer of 3-armed poly(L-glutamic acid)-coupled dopamine or ibuprofen across a novel human co-culture model with induced BBB properties. The dual-targeted nanoformulations of both drugs showed elevated cellular uptake in a time-dependent, active manner via endocytic mechanisms. Free alanine and glutathione inhibited the cellular internalization of targeted nanocarriers suggesting the crucial role of ligands in the uptake processes. The targeted nanocarriers had a higher permeability across the BBB model. After crossing the BBB, the targeted dopamine nanocarriers could subsequently enter midbrain-like organoids derived from healthy and Parkinson's disease patient-specific stem cells. The ibuprofen-coupled targeted nanocarriers had protective effects against cytokine-induced toxicity and BBB opening. These results indicate that coupling dopamine and ibuprofen to BBB-targeted poly(L-glutamic acid) can be used as nanocarriers for nervous system applications.

P35

STREPTOCOCCUS AGALACTIAE EFFECT ON BREAST CANCER RESISTANCE PROTEIN IN BRAIN-LIKE ENDOTHELIAL CELLS

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The blood-brain barrier (BBB) is a network of blood vessels composed of highly specialized brain endothelial cells. The BBB sustains homeostasis in the central nervous system (CNS) by maintaining strict separation between the blood and the CNS. This function is implemented through a collection of tight-junction proteins, efflux transporters, and nutrient transporters. Breast Cancer Resistance Protein (BCRP) is an efflux transporter found at the BBB that effluxes a variety of substrates out of the CNS and into the bloodstream. Group B Streptococcus (GBS) is a bacterium that can bypass the BBB and invade the CNS, resulting in life-threatening inflammation of the CNS referred to as bacterial meningitis. Because GBS is the leading cause of neonatal meningitis, it is extremely pertinent to understand its mechanism of invasion. However, many of the mechanisms that enable GBS to breach the BBB remain unknown, establishing the foundation for this project. We have found through substrate accumulation assays that BCRP substrate accumulation increases during GBS infection, implying that GBS infection hinders BCRP efflux function. Future work will include confirming these results and further characterizing the role of BCRP in GBS infection.

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EFFECTS OF PILT KNOCKOUT ON BLOOD-BRAIN BARRIER INTEGRITY IN VITRO

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The blood–brain barrier (BBB) plays a critical role in maintaining the homeostasis of the central nervous system. Tight junctions (TJs) are dynamic structures composed of a number of transmembrane and membrane-associated proteins arranged in multimolecular complexes and involved in intracellular signaling. Pilt (Protein incorporated later into tight junctions), also known as Tjap1 (Tight junction associated protein 1), is expressed at the BBB and was identified by us as a direct target of microRNA-212/132 under hypoxic conditions. Although the structural composition of TJs complexes has been well described, little is known about the role of Pilt in the properties of the BBB.

In this study, we generated Pilt knockout (KO) endothelial cell lines using genome editing technology. The properties of Pilt KO cell lines were examined by cell viability, tube formation and wound healing assays. To investigate the effects of Pilt on BBB properties in vitro, we measured paracellular permeability, transendothelial electrical resistance (TEER) and performed immunofuorescence staining. To verify the relationship between Pilt and microRNA-212/132, we used microRNA-212/132 KO mice and examined the expression of Pilt in this model.

The Pilt KO cell line showed increased TEER, decreased paracellular permeability, decreased proliferation rate, increased cell migration rate, and altered expression of TJ associated proteins compared the control cell line. In microRNA-212/132 KO mice, expression of its direct target gene Pilt was increased and this was also observed in the transient middle cerebral artery occlusion (tMCAO) model.

In summary, Pilt appears to play an important role in regulating the integrity of the BBB.

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CHARACTERIZATION OF THE APPLICATION POTENTIALS OF MULTICELLULAR SPHEROIDAL HUMAN BLOOD-BRAIN BARRIER MODELS FOR STUDYING THE DEVELOPMENT OF BRAIN-PENETRATING DDS CARRIERS

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[Background] A brain-penetrating drug delivery system (DDS) carrier plays a critical role in delivery of CNS-active drugs to the brain across the blood-brain barrier (BBB). To facilitate the development of the DDS carriers, we have created multicellular spheroidal human BBB models (hMCS-BBB) that show a promising potential for use in BBB permeability evaluation of brain-penetrating DDS carriers. In this study, we aimed to provide further evidence for the utility of the hMCS-BBB models in studies of brain-penetrating DDS carrier.

[Methods] Four types of anti-human transferrin receptor monoclonal antibodies (hTfRMAb) and two types of cyclic peptides (which are models for brain-penetrating DDS carriers) were fluorescently-labeled and used in the BBB permeability assays. The uptake levels in each spheroid were analyzed semiquantitatively by measuring the intraspheroidal fluorescence intensity. The effects of incubation temperature, time, and concentrations on BBB permeabilities were analyzed. [Results] Among the four hTfRMAbs, the three showed significant temperature-dependent BBB permeability. Their BBB penetration were saturable at around 6 hours and 40 μg/mL. As for the peptides, one showed clear BBB penetration ability (the fluorescent level ratio of 37°C/4°C was 9.5-fold), which was greater than that found in the other peptide (2.8-fold). The BBB-permeable peptide showed linear time- and concentration-dependent spheroid uptake profiles, suggesting involvement of a low-affinity transport process.

[Conclusion] We clearly characterized the different BBB permeability properties of the hTfRMAbs and the cyclic peptides using hMCS-BBB models, thereby highlighting the utility of the hMCS-BBB models for characterizing human BBB permeable properties of various brain-penetrating DDS carriers.

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MICROVASCULAR DYSFUNCTION AND CXCL12/PI3K/CREB SIGNALING MEDIATES HIPPOCAMPAL NEUROGENESIS POST- STROKE IN CEREBRAL AMYLOID ANGIOPATHY

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Ischemic stroke and amyloidopathies represent a growing problem within an aging population, underscoring the critical need for research to address these health concerns more effectively. Recovery after an ischemic stroke poses a significant challenge due to sustained tissue damage. Moreover, cerebral amyloid angiopathy (CAA), characterized by substantial Aβ accumulation within the microvasculature, remains poorly understood in the context of post-stroke outcomes. While the brain has a limited ability to recover from stroke through mechanisms like neuroplasticity, the presence of CAA is hypothesized to complicate this recovery. We hypothesized that Aβ accumulation in cerebral microvasculature exacerbates ischemic stroke outcomes and delays post-stroke recovery by inducing BBB dysfunction and aberrant neurogenesis in NPCs. To that end, we used the transgenic 5xFAD mouse model, which mimics Aβ accumulation in the brain, to examine both CAA and cerebrovascular ischemia. Our results showed microvascular dysfunction, decreased perfusion, and delayed functional recovery in mice. We also present the first transcriptional analysis on the mutual effects of CAA and stroke on post-stroke tissue and BBB recovery, revealing distinct transcriptional phenotypes in a subset of NPCs and endothelial cells of the hippocampus. In addition, we demonstrate that NPCs rely on Cxcl12, Pik3c2a, and Creb3l2 signaling for proper neurogenesis post-stroke, which can be targeted for therapeutic recovery. These findings open novel avenues for future research into targeted therapies that may mitigate the impact of CAA and Alzheimer's disease on stroke recovery, offering hope for improved outcomes for individuals suffering from multiple, interconnected neurological conditions.

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STRONG INDUCTION OF IP-10 AND RANTES IN HUMAN BRAIN PERICYTES DESPITE WEAK INFECTION BY TICK-BORNE ENCEPHALITIS VIRUS

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Tick-borne encephalitis virus (TBEV) attacks the central nervous system (CNS) and can lead to severe neurological complications. The neurovascular unit is critical for CNS function and neuroinvasion of TBEV. The specific role of human brain pericytes, a key component of the neurovascular unit, in TBEV infection is still unclear. In this study, TBEV infection in primary perivascular pericytes of the human brain is investigated with the highly virulent Hypr strain and the mildly virulent Neudoerfl strain. Cytokines, chemokines and growth factors were measured using a Luminex assay. Both viral strains showed similar replication kinetics and peaked 3 days post infection (dpi). Intracellular viral RNA levels peaked at 6 dpi for Hypr and 3 dpi for Neudoerfl. Immunofluorescence staining showed that a small proportion of pericytes were infected (3% in Hypr and 2% in Neudoerfl), with no cytopathic effects observed. Production of IL-6 was detected at 3 dpi in the cell culture supernatants, along with a slight increase in IL-15 and IL-4, but IP-10, RANTES and MCP-1 were the predominant chemokines released following TBEV infection. These chemokines are critical for both immune defense and immunopathology during TBE. The results suggest that pericytes are a significant source of these signaling molecules during TBEV infection in the brain.

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PROTECTION OF THE BLOOD-BRAIN BARRIER AS A THERAPEUTIC TARGET IN THE CELL CULTURE MODEL OF ISCHEMIC STROKE

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During ischemic stroke, oxygen-glucose deprivation (OGD) and reoxygenation (OGD/R) cause blood brain barrier (BBB) disruption and neuronal death. Our aim was to protect the endothelial cells of the BBB by improving barrier functions via targeting three signalling pathways with a combination of small molecules (Combination1). We cultured human endothelial cells under different conditions: normoxia, OGD, and reperfusion after OGD (OGD/R) and tested the effect of Combination1 against BBB dysfunctions. Following OGD, the viability of cells significantly decreased, but treatment with Combination 1 ameliorated this effect. OGD and OGD/R condition resulted in reduced transendothelial electrical resistance of the cell layers compared to normoxia, suggesting that paracellular barrier is less tight, but this was significantly elevated by Combination 1 treatment. Penetration of BBB marker molecules elevated after OGD/R suggesting increased permeability of the model, but Combination 1 was able to protect this effect. We investigated whether the protective effect of Combination1 against OGD/R is mediated through an increased expression of a transcriptional regulator of blood-flow response, Krüppel-like factor 2 (KLF2). In contrast, immunocytochemical staining showed that OGD/R increased KLF2 levels compared to normoxia, but decreased after Combination1 treatment. Since the cellular mechanism underlying the protective effect was not linked to KLF2, an important BBB tight junction protein, claudin-5 was investigated. Immunocytochemical staining of claudin-5 was reduced after OGD/R but after Combination1 treatment, the staining was similar to normoxia. Our results suggest that tightening the interendothelial junctions via increasing claudin-5 by Combination1 treatment is a promising approach to protect BBB from post-ischemic injury.

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ENDOTHELIN RECEPTOR ANTAGONISM FOR MODULATION OF BLOOD-BRAIN BARRIER EFFLUX TRANSPORT

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Blood-brain barrier (BBB) efflux transporters are a critical obstacle to the delivery of various drugs for the treatment of central nervous system diseases, particularly anticancer and antiepileptic agents. While the functions and regulation of ABC-family transporters are well-studied, therapeutic strategies to depress BBB efflux function are currently lacking. The endothelin receptors (ET_A and ET_B) are G-protein coupled receptors highly expressed throughout the human cardiovascular system. $ET_{A/B}$ signaling has been linked to the regulation of blood-brain barrier efflux function through multiple mechanisms, including NFκ-B-mediated ABCB1 expression in models of isolated rat brain endothelium and β-Catenin-regulated rat brain expression of Abcb1 and SLC family members *in vivo.* Macitentan is a dual ET A/B antagonist, currently approved by the US FDA and EU EMA for the treatment of pulmonary arterial hypertension. Here, we show that Macitentan, which has a high affinity for both ET_A and ET_B, a long terminal half-life, and pharmacologically active metabolites, is effective in reducing the function of BBB efflux transport in a human brain endothelial cell line, as well as a marked reduction of *in vivo* ABCB1 function as measured by *in situ* brain perfusion.

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MODELING THE MOUSE BLOOD-BRAIN BARRIER: A SIMPLE METHOD FOR GENERATING MOUSE BRAIN ENDOTHELIAL CELL MONOLAYERS

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The function of the blood-brain barrier (BBB) is formed by brain capillary endothelial cells (BECs), presenting a strictly regulated barrier between the blood and the brain. In vitro modeling of the BBB using brain capillary endothelial cells offers an essential tool for drug screening, investigating cell interactions, and predicting transport rates. However, the translation of findings from non-rodent BBB models to preclinical rodent models is hindered by species-specific differences, highlighting the need for a validated mouse BBB model to address these challenges.

The aim of this study was to establish an in vitro BBB model using primary mouse BECs that form a tight monolayer. Cortexes from 4-week-old C57BL/6 male mice were collected, and brain capillary fragments were isolated through a series of steps and then cultivated. After 9 days of cultivation, the isolated BECs were ready for experiments.

Isolated primary mouse BECs were seeded on Transwell supports, forming a monolayer. The identity of the isolated mouse BECs was validated using various techniques, including immunocytochemical staining and gene expression analysis of cell-specific proteins such as von Willebrand Factor (vWF), platelet-derived growth factor receptor beta (Pdgfrβ), and platelet and endothelial cell adhesion molecule 1 (Pecam1). The successful formation of tight junctions was confirmed through immunocytochemical staining, illustrating the junctional location of claudin-5 and ZO-1. Additionally, transendothelial electrical resistance (TEER) measurements and permeability studies using the paracellular transport marker mannitol (180 Da) further validated the formation of tight junctions.

This study demonstrated the successful establishment of an in vitro BBB model using primary mouse BECs cultivated in monoculture. The model formed a tight monolayer with functional tight junctions, and the isolated cells were identified as endothelial cells. The established in vitro mouse BBB models hold promise for developing accurate and translatable preclinical models in central nervous system drug research.

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UTILIZATION OF THE YEAST TWO-HYBRID SYSTEM FOR THE DISCOVERY OF NOVEL TAU INTERACTION PARTNERS

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Proteins are large molecules that play essential roles in all living organisms. Their functions are carried out by interactions with other ligands. The most common interacting molecules of proteins are other proteins, which results into the creation of larger protein complexes. The protein-protein interactions are occurring in all types of cells, and they are playing key roles in both, physiological and pathological processes. Tauopathies are neurodegenerative diseases whose one of the main hallmarks is the formation of neurofibrillary tangles consisting of pathological tau protein forms. Tauopathies include diseases like Alzheimer's disease, Pick's disease and many more. The processes which lead and contribute to tauopathies are currently not sufficiently investigated and understood. Knowledge of the tau protein interaction network is contributing to a better understanding of tau protein's role in both physiological and pathological conditions. Our aim is to find novel tau interaction partners by implementing a cDNA library screening, using the in vivo yeast two-hybrid system. In our project we performed a yeast two-hybrid screening of a human brain cDNA library. The screenings were carried out with the physiological Tau protein and its fragments. We have discovered four novel Tau interaction partners, which are interacting with the N-terminus of Tau protein, and one novel Tau interaction partner interacting with the full-length Tau protein. Three of the five novel Tau interaction partners have been additionally validated by co-immunoprecipitation and co-localization experiments.

Keywords: Protein-protein interactions, Tau protein, Alzheimer's disease, yeast two-hybrid system

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CORTICAL PLASTICITY IS ASSOCIATED WITH BLOOD-BRAIN-BARRIER MODULATION

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Blood-brain barrier dysfunction (BBBd) and its role in brain diseases has been widely studied. In models of brain injury, BBBd dysfunction led to extravasation of serum albumin to the brain and activation of transforming growth factor beta (TGF-b) signaling, result with neruoinflammation, pathological plasticity, neuronal hyper-excitability, and seizures. Recent works support evidences for regulation of BBB permeability by circadian rhythm, suggest for physiological modulation of the BBB. However, the effect of neuronal activity on BBB function in the healthy brain and whether it plays a role in brain plasticity is still unclear. In the present animal and human study we show that neuronal activity, in the physiological range, induces focal modulation of BBB permeability in the healthy brain and that it has a role in local network reorganization. By combining in vivo extracellular recording, direct fluorescent microvascular imaging and transcriptomic analysis in rats with human data-based functional and BBB-mapping MRI, we show that prolonged activation of the limb induces a focal modulation of BBB permeability in the corresponding somatosensory cortex that is associated with long-term synaptic plasticity. We further show that this modulation of microvascular permeability depends on neuronal activity and involves caveolae-mediated transcytosis and TGF-b signaling. Our results reveal a role of BBB modulation in cortical plasticity in the healthy brain, highlighting the importance of neurovascular interactions for sensory experience and learning.

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A PERSONALIZED AND BIOMIMETIC MODEL OF BLOOD BRAIN BARRIER TO EXPLORE AUTOLOGOUS LEUKOCYTES MIGRATION IN MULTIPLE SCLEROSIS

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Progressive BBB breakdown and infiltration of autoreactive leukocytes into the CNS are central features in the pathogenesis of neuroinflammatory disorders, such as Multiple Sclerosis (MS).

To develop a humanized NVU in vitro model for addressing MS pathogenic molecular mechanisms, we exploit circulating endothelial colony forming cells (ECFCs) isolated from the peripheral blood of MS patients and healthy subjects as a source of primary endothelial cells, that are co-cultured with primary human astrocytes growing on opposite sides of a matrix-coated permeable membrane.

The endothelial identity of ECFCs was characterized by multi-color flow cytometry and confocal imaging, confirming the expression of endothelial epitopes and their ability to uptake acetylated-LDL. Cultured ECFCs, under pro-inflammatory conditions, upregulated adhesion molecules, a key feature to study the phenotype of T cell subset specifically transmigrating in MS. Also, ECFCs were co-cultured with human astrocytes, and the integration into the NVU platform ameliorated barrier properties. Autologous lymphocytes are used to perform subject-specific transmigration studies across the NVU model. We evidenced a different transmigration capacity of T cells isolated from the same person with MS (pwMS) when treatment-naïve relapsing and at remitting follow up stage. Moreover, we set up and validated a multicolor flow cytometry panel to immunophenotype the signature of T cells transmigrating across the in vitro model in a prospective cohort of pwMS and controls.

Thus, we propose our NVU model as a human disease-relevant tool to investigate autologous leukocytes migratory capacity and immunophenotype in pwMS.

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TRANSPORT CHARACTERISTICS OF A BLOOD-BRAIN BARRIER-PERMEABLE CYCLIC PEPTIDE-FUSED MONOCLONAL ANTIBODIES IN IN VITRO BLOOD-BRAIN BARRIER MODELS

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Monoclonal antibodies (mAbs) are promising therapeutic agents for various central nervous system diseases; however, their delivery to the brain is limited by the blood–brain barrier (BBB). We previously identified a BBB-permeable cyclic peptide (SLS peptide) that enhances macromolecular transport across the BBB. In this study, we aimed to investigate the BBB permeability of SLS peptide-fused mAbs using *in vitro* BBB models. Trastuzumab (TCH) was used as the model human mAb to generate the TCH-SLS antibody, in which the SLS peptide was fused to the C-terminus of TCH via a G4S linker. To determine the optimal G4S linker length for this fusion, we generated TCH-H-(G4S)x-SLS1 using G4S linkers of different lengths (x = 1, 3, and 9). Uptake studies in hCMEC/D3 cells revealed that the TCH-fused SLS peptides generated using three G4S linkers enhanced the TCH cellular internalization. Furthermore, permeability assay in a rat BBB co-culture transwell model revealed the significantly higher permeability of TCH-H-(G4S)3-SLS1 across the BBB than TCH alone. Another permeability assay in a human immortalized multicellular spheroidal BBB model confirmed that TCH-H-(G4S)3-SLS1 exhibited significantly higher permeability across the BBB than TCH alone. To assess the pharmacological effects of TCH-H-(G4S)3-SLS1, cell viability assays were performed using BT-474 (HER2-positive breast cancer) cells. Cytotoxic effects of TCH-H-(G4S)3-SLS1 on BT-474 cells were comparable to those of TCH alone. In conclusion, our results suggest that fusion with the SLS peptide enhances the BBB-permeability of mAbs without altering their pharmacological effects.

P47

HIV ALTERS GAP JUNCTION COMMUNICATION IN LATENTLY INFECTED BLOOD-BRAIN BARRIER PERICYTES

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The use of anti-retroviral therapy has significantly reduced the morbidity and mortality of HIV-1 disease progression, yet HIV-Associated Neurocognitive Disorders (HAND) persist with over 50% of patients experiencing mild to severe degrees of cognitive decline. Recent studies suggests that HIV-1 can disrupt communication between cells of the blood-brain barrier (BBB), potentially contributing to HAND development. Pericytes, crucial for maintaining homeostasis of the cerebrovascular microenvironment, can be productively infected with HIV-1. Given their pivotal role and susceptibility to HIV-1, we hypothesize that HIV-1 infection of pericytes disrupts BBB endothelial cell integrity through dysfunctional signaling via gap junctions and hemichannels, potentially exacerbating HAND development. To this aim, we employed a co-culture model with primary human BBB pericytes and microvascular endothelial cells in which pericytes were infected with HIV-1 for 3 or 7 days exhibiting active and latent viral phenotypes. Analysis of co-cultures confirmed a dysregulation of BBB integrity in both actively and latently infected pericytes. Furthermore, latently infected pericytes showed an upregulation of connexin and pannexin genes. Ongoing functional studies of gap junction and hemichannel signaling will determine if these channels are used to transmit injury signals from infected to no-infected cells. Further studies on the cellular and molecular mechanisms driving the pathogenesis of HAND could provide potential targets for future treatments and interventions.

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P48

DOES ALTERED OF EXPRESSION OF TJPS ACCOUNT FOR BBB LEAKINESS IN THE PRECUNEUS IN AD?

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Introduction:

Monolayer endothelial cells, interconnected by apically localised tight junction proteins (TJP), form a physical barrier, known as the blood-brain barrier (BBB) that controls molecular exchange between the blood and the brain. BBB breakdown occurs in the early-stage Alzheimer's disease (AD) and contributes to cognitive decline and disease progression. In this study, we examined whether the expression of TJPs, claudin-5 and occludin, were altered in relation to Braak tangle stage (BS) in microvessel-enriched fractions (MVFs) of the precuneus within the parietal cortex.

Materials and Methods

We studied 60 brains – BS 0-II (n=17); III-IV (n=23); V-VI, (n=20) – from the South-West Dementia Brain Bank, University of Bristol. MVFs were homogenised to measure claudin-5 and occludin levels by in-house ELISAs. MVFs from 29 brains – BS 0-II, n=15; V-VI, n=14 –were also fixed and immunolabelled for claudin-5 or occludin, and the mean pixel intensity levels were compared.

Results

The levels of claudin-5 and occludin were similar in BS 0-II and V-VI brains but claudin-5 level tended to be higher in BSIII-IV than in BSV-VI brains ($p=0.06$); this difference reached significance after claudin-5 level was normalised to vessel content (CD31 level) ($p=0.04$). Analysis from Immunofluorescence labelling also showed that relative level of claudin-5 and occludin did not alter between BS0-II and BSV-V-VI.

Conclusion:

Our findings indicate that breakdown of the BBB in the precuneus in early AD is unlikely to be caused by loss of TJPs. ELISA and immunofluorescence data show that claudin-5 and occludin level remains unchanged during the course of disease.

P49

DYNAMIC CONTRAST-ENHANCED MRI REVEALS BLOOD-BRAIN BARRIER DYSFUNCTION IN NEUROLOGICAL DISORDERS: INSIGHTS FROM A MULTI-CENTER STUDY

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Dysfunction of the blood-brain barrier (BBBD) has been implicated in various neurological disorders, including traumatic brain injury, epilepsy, and neurodegenerative conditions. We summarize our experience with over 800 dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) scans from healthy controls and patients with different neurological conditions in four different medical centers. Data from 100 healthy controls (ages 18-80) revealed age- and region-dependent variations in BBB permeability. In a cohort of 50 patients with drug-

resistant epilepsy (DRE), BBBD was observed in the majority of patients. BBBD was evident in brain regions suspected to be involved in seizure onset in 66.6% of patients (N=39) and/or in the same hemisphere as the suspected epileptogenic lesion. In patients with neurological disorders such as bipolar disorder, mild cognitive impairment, and mild traumatic brain injury a leaky BBB was detected in 25-50% of patients when compared with healthy controls. BBBD could be either focal or diffuse. Diffuse BBBD was associated with more severe symptoms and cognitive decline. These findings are consistent with preclinical studies that underscore the role of BBBD in neurological disorders and suggest DCE-MRI as a valuable diagnostic and potentially pharmacodynamic biomarker.

P50

TRAUMATIC BRAIN INJURY INDUCES SENESCENCE IN CELLS OF THE NEUROVASCULAR UNIT

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Traumatic brain injury (TBI), is one of the leading causes of disability. It induces vascular damage, accelerates cellular senescence, inflammaging, cognitive aging, and impairs repair. Cellular senescence caused by double-stranded DNA damage was identified as the mechanism driven by brain dysfunction after TBI.

We quantified TBI-induced senescence in the neurovascular unit using the Marmarou weight drop model, in 4 groups of rats: severe TBI (STBI), mild TBI (MTBI), repetitive mild TBI (rMTBI), and control (rSHAM) at two time points: 24 hours and 4 weeks. Brain sections were co-stained with a senescence-specific marker (γH2AX) and cell type-specific markers (CD31, GFAP, Iba1, and PDGFRβ for endothelial cells, astrocytes, microglia, and pericytes) and imaged using epifluorescence and laser confocal microscopy.

A statistically significant number of astrocytes were γH2AX positive in STBI after 24 hours. Microglia were γH2AX positive in statistically significant numbers in STBI and MTBI after 24 hours. Senescent astrocytes and microglia disappeared after 4 weeks. No γH2AX positive pericytes were found in any of the experimental groups. Senescent endothelial cells appeared in response to TBI only after 4 weeks but this was only statistically significant in the cortex in STBI. Behavioural testing found no significant changes.

These data suggest a minor involvement for brain microvasculature in TBI induced senescence.

P51

METHAMPHETAMINE ACTIVATES INFLAMMATION AND NEURODEGENERATIVE CHANGES IN C57BL/6 MICE BYPASSING THE NLRP3 INFLAMMASOME.

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Methamphetamine (METH) is known to impair the dopaminergic system by increasing the release of both dopamine and serotonin from nerve endings in the central nervous system. The above actions affect cells in the hippocampus, causing activation of inflammation in people chronically exposed to METH. However, it is not clear whether the activation of inflammation by METH is mediated solely by the NLRP3 inflammasome or whether there are alternative inflammasomes that activate inflammation in the hippocampus, resulting in impaired cognitive abilities. We addressed these emerging problems by blocking the NLRP3 inflammasome using the pharmacological inhibitor MCC950. Mice were injected with METH three times per day for 5 days in ascending doses (starting with 0.2 mg/kg to the final dose of 2.4 mg/kg) using a step-wise increase of 0.2 mg/kg with each injection. Then, the mice were exposed for one more day to a high-dose METH binge based on three successive injections of 4.0 mg/kg METH at 4 h intervals. Moreover, on each day of METH exposure, mice were injected with the MCC950 inhibitor at 20 mg/kg. Inflammatory responses were evaluated using the Bio-Plex Pro Mouse Cytokine 23-plex Assay. Neurogenesis disturbances in the hippocampus were assessed by immunofluorescence methods. The measurement of cognitive abilities was performed using the NOR and MWM tests. The obtained results showed an alternative pathway of inflammation activation, independent of the NLRP3 inflammasome, which negatively influenced cognitive abilities. The research was financed by the National Science Centre, grant no. 2019/33/B/NZ4/02721.

P52

CHRONIC INSTABILITY STRESS ALTERS BLOOD- BRAIN BARRIER COMPONENTS AND BEHAVIOUR OF ADULT FEMALES RATS IN EXPOSITION TIME MANNER

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Chronic stress is recognized as a one of the risk factors for several psychiatric disorders. In patients suffering from cardiovascular disease the prevalence of developing depression is two- to three-times higher. Moreover, dysfunctions at the blood-brain barrier (BBB) level seems to be relevant to emergence of depression-like behaviours in animal models, thus linking stress and BBB dysfunction as a combined cause of neuropathology.

The aim of the study was to investigate if time of exposition to chronic instability stress (CSIS) may influence animals behaviour and BBB integrity and consequently determine susceptibility or resilience to chronic diseases. Therefore, adult female Sprague-Dawley rats were subjected to the 6- and 12-weeks CSIS procedure involving unpredictable rotation between phases of isolation and overcrowding. After that time their behavioural phenotype was evaluated by open field (OFT) and novel object recognition tests. Moreover, the protein levels of selected BBB components were qualified to assess its integrity in the prefrontal cortex.

We observed that the CSIS induced cognitive decline regardless of the duration. However, after 6-weeks of CSIS did not caused significant changes in female behaviours measured in the OFT, except enhanced rearing behaviour. While, after 12-weeks of CSIS, locomotor activity and grooming behaviour were significantly blunted. At the level of BBB, CSIS significantly altered the cortical protein levels of Zona-Occludens1 and Occludin after 6-weeks, but not 12-weeks of stress exposition.

These results suggest that CSIS procedure determine the females' behavioural phenotype, to less active with the time, and concomitant progressive adaptation changes at the BBB level.

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P53

DOSE-DEPENDENT INCREASE IN CXCL10 IN BRAIN ENDOTHELIAL CELLS AND GLIA FOLLOWING PERIPHERAL INFLAMMATION: POSSIBLE BIOMARKER FOR CEREBROVASCULAR INJURY?

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Introduction: Neurovascular inflammation recognised as an early feature of neurodegenerative disease. A circulating biomarker that indicates these early changes would enhance diagnosis and treatment. Here we aimed to identify and evaluate possible inflammatory biomarkers that may be secreted from endothelial cells following activation.

Methods: RNAseq datasets of endothelial transcription following inflammatory injury were analysed using Ensemble of Gene Set Enrichment Analysis. Male and female CD1 mice received a 0.33 or 1 mg/kg i.p. injection of lipopolysaccharide (LPS; E. coli 0111:B4) or saline and brains were collected and processed for histology 4 hours after treatment. Sections were stained for blood vessels, plasma proteins, microglia, astrocytes, and the chemokine CXCL10. Animal experiments were approved by the RVC Animal Welfare and Ethical Review Board and performed under home office licence PDADGE285.

Results: A number of inflammatory genes were found to be high transcribed 15 minutes to 4 hours after stimulation. CXCL10 was the most consistently altered across paradigms and remaining up-regulated until 72 hours. A dose-dependent increase in CXCL10 protein was detected in the brain of mice following LPS exposure, with protein present in endothelial cells and glia. No break-down of the blood brain barrier was detected with these levels of inflammatory stimulus, or within the investigated timeframe. There were no sex-differences detected in this response.

Conclusions: CXCL10 is consistently up-regulated for hours after injury in a dose-dependent fashion in endothelial and glial cells. As a secreted protein detectible in the plasma, it may, therefore, be a possible biomarker of general neuroinflammation.

P54

IN VIVO DISTRIBUTION OF ELETRIPTAN AND SUMATRIPTAN WITH LINKAGE TO PREDICTED TARGET ENGAGEMENT IN THE CNS AND PNS

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Background: Triptans are potent 5-HT1B/1D receptor agonists used in antimigraine therapy. Strong evidence implies that triptans mediate its antimigraine effect through peripheral mechanisms of action. Additionally, an existing question concerns whether triptans cross the blood-brain barrier (BBB) to a degree that contributes to their antimigraine effect via stimulation of central 5-HT1B/1D receptors. The aim of this study was to explore eletriptan and sumatriptan disposition in different regions of the CNS and PNS, with the overall goal of predicting 5-HT1B/1D receptor engagement at therapeutically relevant plasma concentrations.

Methods: This study employed a Combinatory Mapping Approach (CMA) to assess unbound drug concentrations in CNS and PNS regions in rats. The CMA integrates preclinical neuropharmacokinetic studies with in vitro brain slice and equilibrium dialysis experiments to determine unbound brain-to-plasma concentration ratios (Kp,uu). These ratios, along with in vitro 5-HT1B/1D receptor potencies, were used to predict target engagement in the CNS at clinical plasma concentrations.

Results: We show heterogeneity in the extent of eletriptan and sumatriptan transport across the BBB and blood-nerve barrier. The highest drug exposure was observed in the trigeminal ganglion with Kp,uu values close to unity. Both eletriptan and sumatriptan entered the brain parenchyma with Kp,uu values of 0.06 and 0.05, respectively. Despite low BBB penetration, mathematical predictions indicate that both triptans achieve unbound brain concentrations sufficient for central target engagement.

Conclusion: Eletriptan and sumatriptan may stimulate central receptors at clinically relevant conditions, suggesting a central mechanism of action in addition to known peripheral effects.

P55

INVESTIGATION OF DEXAMETHASONE LOADED POLYMER MICELLES ON THE CULTURE MODEL OF NASAL EPITHELIUM AND THE BLOOD-BRAIN BARRIER

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The blood-brain barrier (BBB) prevents the passage of biomolecules from the blood to the brain. Consequently, the effective treatment of neurodegenerative diseases has been a challenge. Intranasal administration can occur as an alternative drug delivery route to the brain, that allows a rapid solution to bypass the BBB where the intact drug can penetrate through the nerval pathways directly into the brain. Another problem is that potential drug candidates cannot access the brain in a therapeutically relevant concentration. Hence, novel drug delivery systems are needed. Polymeric micelles can offer increased solubility, enhanced drug release and permeability through biological barriers. In our study, we investigated the effect and penetration of dexamethasone, a steroidal anti-inflammatory drug with poor water solubility, encapsuled into polymeric micelle on the human culture model of the nasal epithelium and the BBB. Real-time impedance measurement showed a slight decrease in cell index of the nasal epithelial and the brain endothelial cells after 30- and 60 minutes of the treatment. We observed increased penetration of the dexamethasone loaded polymeric micelle across the nasal epithelial and the brain endothelial cell layer compared to the dexamethasone suspension treated group at 30- and 60-minute timepoints. After the permeability experiment, we tested the cell layer integrity by measuring the transendothelial- and epithelial electrical resistance and the penetration of two different marker molecules. Based on our results, polymer micelles can be safely used to enhance the permeability of dexamethasone through the nose into the brain.

P56

THE HISTONE DEACETYLASE INHIBITOR SUBEROYLANILIDE HYDROXAMIC ACID PROMOTES BLOOD-BRAIN BARRIER PROTECTION DURING REPERFUSION IN A CELL CULTURE MODEL OF ISCHEMIC STROKE

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Ischemic stroke is a leading cause of death worldwide with limited available treatment options. During ischemic stroke, reduced blood flow causes severe neuronal damage and BBB disruption. Thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) which acts by breaking down clots to restore blood flow are the only FDA approved treatment for ischaemic stroke. However, rtPA is effective within a limited time (4.5 h) of symptom onset with a risk of intracranial hemorrhage. Therefore, additional effective therapeutic approaches for treatment of ischaemic stroke are urgently needed. The aim of this study was to investigate the effect of suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, on restoring the function of the BBB to prevent post-stroke consequences.

We tested the effect of SAHA on human co-cultured BBB model under normoxia, oxygen-glucose deprivation (OGD), and during reperfusion after OGD (OGD/R). Our results show that after OGD, SAHA is able to prevent the BBB functions by increasing the resistance, and reducing the permeability of marker molecules across the BBB. SAHA increased the viability of pericyte cells against injury, which were much more damaged than brain endothelial cells. RNA-seq analyses showed that SAHA decreased the expression of genes involved in inflammatory processes and cell proliferation and increased the expression of the genes of glycocalyx enzymes.

Based on our results, SAHA may be a potential therapeutic tool for the treatment of ischaemic stroke, but further research is needed to gain a deeper understanding of its mechanism of action and to develop its potential clinical applicability. A.S. was supported by Gedeon Richter Talentum Foundation. S.V. was supported by the Young Researcher Excellence Program (FK 143233) by National Research, Development and Innovation Office of Hungary. G.P. was supported by the National Academy of Scientist Education (FEIF/646-4/2021-ITM_SZERZ).

P57

BIODISTRIBUTION OF MODIFIED ISCHEMIC STROKE NEUROPROTECTIVE TAT-NR2B9C PEPTIDES UNVEILED A SEX SPECIFIC BRAIN UPTAKE FAVORING FEMALE BRAINS

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Globally, stroke is the second most common cause of death. There are two main types of strokes: ischemic and hemorrhagic where ischemic stroke accounts for over 80% of all the cases. An ischemic stroke occurs when a blood vessel supplying the brain becomes clogged resulting in initiation of a downstream pathological cascade termed excitotoxicity; ultimately leading to neuronal death. Currently, only one drug for treating ischemic stroke is approved, i.e. the thrombolytic agent rh-tPA, while excitotoxicity interfering drugs are completely lacking. Nevertheless, an excitotoxicity interfering drug called, Tat-NR2B9c (also known as NA-1 or nerinetide) has shown promising pre-clinical therapeutic potential. However, it failed in a phase III clinical trial due to instability and off-target biodistribution. However, if these challenges are tackled, the field may witness the first excitotoxicity interfering drug to be approved for treating ischemic stroke.

To address this issue, we modified Tat-NR2B9c by converting from L- to D-amino acids, including selenium-methionine for detection, and replacing Tat with different brain homing peptides; namely a cell-penetrating peptide (SynB3), a transferrin receptor targeting peptide (T7), and a stroke homing peptide (SHP). Biodistribution of the peptides was assessed by inductively coupled plasma mass spectrometry, and their binding affinities to their neuronal targets (PDZ1 and PDZ2 domains) were investigated with surface plasmon resonance. The modified Tat-NR2B9c peptides were all detected in the brain one hour post intravenous administration (~2-4 %ID/g tissue, highest brain uptake was observed for D-, L-SynB3-NR2B9c, and D-Tat-NR2B9c). Interestingly, Tat-NR2B9c modified with T7, D-SynB3 and L-SHP showed a sex-dependent brain uptake favoring female brains. Eventually, the modified Tat-NR2B9c peptides require testing in ischemic stroke animal models to ensure that they provide the critical neuroprotection. Additionally, the data also stresses the importance of including both sexes in preclinical research.

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EFFECTS OF HSPB1-OVEREXPRESSION ON INFLAMMATION AND GLIAL ACTIVATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Heat shock proteins (HSPs) are evolutionarily conserved chaperones. Previously we observed that overexpression of HSPB1 improved certain symptoms of Alzheimer's disease (AD) in mice. The primary role of these chaperones is to maintain protein homeostasis, however, several evidence suggest that they have additional functions, such as regulating inflammation. Therefore, we aim to investigate the role of HSPB1 in neuroinflammation and glia activation in a mouse model of AD.

AD model (APP/PS1) mouse strain was crossed with an HSPB1 overexpressing line. Gene expression levels of cytokines and glia activation markers were investigated by qPCR in the hippocampus of 12-month-old animals. Additionally, morphological alterations and plaque-associated accumulation of glial cells were examined through immunohistochemical stainings. Due to a high incidence of seizures, female AD model mice show a higher mortality rate, which was remarkably reduced by HSPB1 overexpression. Immunofluorescent staining confirmed the accumulation of HSPB1 and activated glial cells around the Aß plaques. In the APP/PS1 group, the expression levels of IL-1ß, IL-6, TNFα, astrocyte, and microglia marker genes were significantly higher than those in wild-type animals. However, HSPB1 overexpression led to increased expression of M2 anti-inflammatory microglia markers in APP/PS1 mice.

Our results confirm that HSPB1 has protective effects against the symptoms of AD, as evidenced by the lower mortality rate of APP/PS1/HSPB1 mice. Furthermore, overexpression of HSPB1 may influence the activation of the microglia cells promoting tissue repair.

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EFFECTS OF CONTRAST AGENTS USED IN MEDICAL IMAGING ON THE BLOOD-BRAIN BARRIER

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The use of contrast media (CM) during cerebrovascular surgeries has rapidly increased. During neurointervention computer tomography revealed extravasation of the CM across the blood-brain barrier (BBB) to extracellular spaces of the brain parenchyma. The aim of this study was to investigate the effect of CM on BBB integrity, since the impact of CM extravasation and the related side effects still remain controversial.

We established an *in vitro* BBB co-culture model using rat primary endothelial cells, astrocytes and pericytes. Two types of CM were tested: iodixanol, an isoosmolar agent and iopamidol, a hyperosmolar agent. To evaluate the effect of CM on BBB functions 30 minute treatment was used and the recovery of the cellular monolayers was examined for 24 hours by real time cell analysis. Barrier integrity was evaluated using the primary triple co-culture BBB model by measuring transendothelial electrical resistance (TEER), permeability and by analysing tight junction morphology.

Short term direct CM exposure caused a significant drop in the impedance of the brain endothelial monolayer without the possibility of recovery. 1% and 10% treatment concentrations also decreased the barrier integrity, but these groups recovered to the level of the control after 24 hours. Junctional morphology, resistance and permeability studies also showed a disturbance in barrier integrity.

Extravasation of CM across the BBB raises awareness to the negative impact of neurointervention and endovascular therapy. It also shows that the clinical outcome for patients with acute ischemic stroke depends on the approach how CMs are used in everyday practice. The work was supported by the HAS-JSPS Bilateral Grant agreement. J.P.V. is supported by the Gedeon Richter Talentum Foundation.

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ESTROGEN SIGNALING CONTRIBUTES TO GROUP B STREPTOCOCCAL DISRUPTION AND INVASION OF BRAIN ENDOTHELIAL CELLS

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Bacterial meningitis is a serious life-threatening infection of the central nervous system (CNS) that occurs when blood-borne bacterial pathogens can disrupt the blood-brain barrier (BBB) and enter the CNS. The BBB is comprised of highly specialized brain endothelial cells (BEC) that serve to protect the CNS from toxins and pathogens while supporting proper brain function. Group B Streptococcus (GBS) is the leading cause of neonatal meningitis and mechanisms of how the BBB fails to protect the CNS during infection remain unclear. We have conducted microRNAseq on BECs either infected with GBS or mock infected and we found that globally microRNAs are downregulated. Estrogen signaling has been demonstrated to contribute to global microRNA downregulation and we hypothesize that estrogen signaling may play a role in GBS – BEC disruption. Our preliminary findings demonstrate that treatment of BECs with estrogen receptor (ER) antagonists is sufficient to inhibit GBS invasion of BECs and rescue candidate microRNA expression. Additionally, we find that BECs treated with the ER agonist beta-estradiol is sufficient to reduce microRNA expression and increase rates of bacterial invasion. Our findings suggest that GBS may utilize estrogen signaling to gain access to the CNS and cause bacterial meningitis. Future work will elucidate mechanisms of global microRNA failure and determine if rescue of microRNAs can restore BBB function during GBS infection. Additionally, we will determine if GBS induces endogenous ER signaling. We show for the first time, that ER signaling can contribute to GBS invasive disease.

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METHAMPHETAMINE NEGATIVELY AFFECTS THE FUNCTIONING OF MITOCHONDRIA IN ASTROCYTES.

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Methamphetamine (METH) abuse is a major public health issue around the world, yet there are currently no effective pharmacotherapies for the treatment of METH addiction. The continuous use of METH eventually leads to drug addiction and causes serious health complications, including memory loss and cognitive decline. These neurological complications are strongly associated with METH-induced neurotoxicity and neuroinflammation, which leads to neuronal cell death.

From the generation of neural stem cells to the maintenance of neurons and their ultimate demise, mitochondria play a critical role in regulating neural pathways' homeostasis, a task that is critical to the cognitive health and neurological well-being. Mitochondria provide energy via oxidative phosphorylation for the neurotransmission and generation of an action potential along the neuron's axon. Exposure to METH promotes i.e. mitochondrial dysfunction and oxidative stress.

The aim of the study was to analyze the impact of METH on oxidative phosphorylation and glycolysis in an established astrocyte (C8D1A) cell line.

Mitochondria functionality was analyzed in cells incubated with different doses of METH (0.1–2.0 mM) using the Real-Time ATP Rate Assay and Mito Stress Test in the Seahorse system (Agilent).

The obtained results show that METH at a dose of at least 0.5 mM affects the level of oxygen consumption rate and extracellular acidification, which results in poorer functioning of mitochondria.

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BLOOD-BRAIN BARRIER INTEGRITY SAFEGUARDED BY EXOGENOUS NICOTINAMIDES IN OXIDATIVE STRESS

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Oxidative damage is primarily caused by an imbalance between the production of reactive oxygen species (ROS) and the cell's ability to detoxify intermediates or repair the resulting damage. ROS-induced oxidative damage can compromise the integrity of the blood-brain barrier (BBB) and is associated with various neurological disorders. One of the cellular components of the antioxidant defense system is the coenzyme nicotinamide adenine dinucleotide (NAD+) in redox reactions, present in several precursor forms. Nicotinamide, one of the precursors is known to counteract elevated oxidative stress. Therefore, the bioactivity of selected nicotinamide derivates (NAs) was evaluated as a possible safeguard of BBB from oxidative stress. As a model, human brain endothelial cells (hECs) were used to check viability, (anti)oxidative parameters and integrity of the BBB. All tested NAs were not toxic, although one NA increased cell impedance. Further, it protected hECs by reducing ROS and returning activity of glutathione, mitochondrial membrane potential and superoxide dismutase to a basal level. After oxidative stress, one of the NAs (with methoxy moiety) increased transendothelial electrical resistance and claudin-5 level and decreased permeability. These results warrant further studies to investigate in detail the exact mechanism of the observed BBB protection, to understand the key factors are involved in this process.

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FLUIDFM AND RESONANT WAVEGUIDE GRATING OPTICAL BIOSENSORS AS VALUABLE TOOLS IN BLOOD-BRAIN BARRIER RESEARCH

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With today's single-cell techniques, we can explore tissues at a cellular level, deepening our understanding of cellular heterogeneity and identifying potential subpopulations. This knowledge can lead to novel health and medical applications. Notable techniques include resonant waveguide grating-based label-free optical biosensors (RWG) and fluidic force microscopy (FluidFM). FluidFM uses a hollow, microfabricated cantilever connected to a liquid reservoir and pressure controller, allowing precise, gentle interaction with individual cells.

Understanding the blood-brain barrier (BBB) physiology and maturation is crucial as it affects drug delivery to the brain and is linked to neurological diseases. We measured the adhesion force of human vascular endothelial cells using cantilevers with 8 µm circular openings. RWG optical biosensors enabled real-time measurement of cell adhesion and spreading by detecting local refractive index changes near the sensor surface.

We treated endothelial cells with a molecular combination (cARLA), which targets three signalling pathways and induces BBB properties in several types of culture models. The treatment (24 h) strengthened the intercellular junctions, allowing us to measure mature adhesion forces with FluidFM more effectively. The force-distance curves showed two significant minima, correlating with adhesion bond distribution observed via fluorescence microscopy. The results of the two techniques (FluidFM and RWG biosensor) were consistent regarding the impact of cARLA treatment on the mechanical integrity of the BBB. Our work demonstrated that the RWG biosensors and FluidFM are valuable biophysical techniques in cell adhesion studies and in BBB research.

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IN VIVO EFFECT OF NOVEL PERIPHERALLY RESTRICTED AND MODERATELY BRAIN-PENETRANT CANNABINOID RECEPTOR 1 MODULATORS IN ALCOHOL USE DISORDER

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Cannabinoid receptor 1 (CB 1 R) antagonism holds therapeutic potential in the treatment of several disorders, such as addictive disorders. Novel pharmacological tools to investigate allosteric/dualsteric strategy could aid mechanistic understanding of functional selectivity in CB 1 R that eventually help developing effective and safer therapies. Accordingly, wedesigned, synthesized, and evaluated a series of novel compounds in the in vitro and in vivo assays.

Novel compounds with high affinity and selectivity for CB 1 R in the sub- and low nanomolar range were tested in functional assays using [35 S]-GTPγS binding. The tested compounds retained high potency for CB 1 R antagonism. Two of the tested compounds behaved as non-competitive CB 1 R antagonist in GTPγS binding with Schild plot analysis indicating negative allosterism. In pharmacokinetic studies, the tested non-competitive antagonists provided good systemic exposures in 3 mg/kg intraperitoneal injections. Acute treatments with enantiomerically pure compounds at 3 mg/kg dose provided maximum in vivo efficacy for CB 1 R antagonism with fully attenuating CB 1 R agonist effect in upper GI motility assay. Compound-1 was peripherally restricted with 8% brain/plasma ratio whereas compound-2 was moderately brain-penetrant with 34% brain/plasma ratio. Unlike rimonabant (10 mg/kg), neither of the two tested compounds (10 mg/kg) induced hyperambulatory activity. Additionally, both compounds dose (1, 3, 10 mg/kg) dependently reduced alcohol drinking in DID experimental paradigm.

We developed peripherally restricted or moderately brain-penetrant bitopic CB 1 R modulators with favorable pharmacokinetics and potent in vivo efficacy, without causing anxiogenic effects. Further studies are needed to characterize these compounds in models and paradigms, demonstrating in vivo selectivity and improved CNS safety.

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