3rd UK and Ireland Early Career Symposium 22nd November 2013

MedImmune, Milstein Building, Granta Park, Cambridge, UK

Registration, refreshments and poster set up (10:15 to 11:00)

Session 1 (11:00 to 12:45)

Introduction to the day and opening remarks

Iain Chessell (VP Neurosciences, MedImmune) Key note presentation "The promise of crossing the blood brain barrier: challenges and progress."

Mohd Hamzah Mohd Nasir (Keele University) "The secondary effect of Plasmodium falciparum infected red blood cells (PRBC) sequestration on blood-brain barrier (BBB) permeability in cerebral malaria (CM) - an in-vitro study"

Ursula Püntener (University of Southampton) "Contribution of the blood brain barrier (BBB) in neuro-immune communication following bacterial infection"

Nichola Fletcher (Birmingham University) "CD4+ T cells and inflammatory cytokines potentiate Hepatitis C virus infection of the BBB"

Lunch and posters (12:45 to 13:45)

Session 2 (13:45 to 15:05)

Dongsheng Wu (Open University) "Brain endothelial miR146a inhibits leucocyte adhesion through targeting NFAT5"

John Connell (Oxford University) "Selective permeabilisation of the blood-brain barrier at sites of metastasis"

Fionn O'Brien (University College Cork) P-glycoprotein inhibition as a strategy to augment antidepressant delivery across the blood-brain barrier: preclinical pharmacokinetic and pharmacodynamic studies

Marie O'Connor (Institute of Ophthalmology) "Regional heterogeneity in the endothelial glycocalyx and basement membrane of the retinal vasculature"

Refreshments and posters (15:05 to 15:30)

Session 3 (15:30 to 17:00)

Radka Gromnicova (Open University) "Small gold nanoparticles: potential carriers of therapeutics across the blood-brain barrier"

Julie Wang (Kings College, London) "Chemically functionalised carbon nanotubes as potential vectors for brain delivery in vivo"

Gavin Fullstone (UCL) "Modelling the Transport of Nanoparticles across the Blood-Brain Barrier"

Optional talks

Dinesh Chauhan (The Right Thing) "Making the transfer from Academic to Industry: Success in the recruitment process." An insight into some of the key differences in approach when seeking an industrial role vs. academic. Including CV construction and identification of opportunities.

Lutz Jermutus (Senior Director, MedImmune) "Careers in industry"

Lab tours, networking and drinks (16:30 to 18:00)

Transport home, or retire to local hostelry for further networking (18:00 onwards)

Talk Abstracts

Mohd Hamzah Mohd Nasir, Srabasti J. Chakravorty and Monique F Stins*

The secondary effect of *Plasmodium falciparum* infected red blood cells (PRBC) sequestration on blood-brain barrier (BBB) permeability in cerebral malaria (CM) - an *in-vitro* study.

Institute for Science and Technology in Medicine, School of Life Sciences, Keele University, Keele, Staffordshire, ST5 5BG, UK. * John Hopkins University, Baltimore, USA

Sequestration of PRBC in the lumen of brain microvessels is one of the hallmarks for CM. This complication can be fatal, and in cases of recovery, long-term neurological defects are seen. Post-mortem examinations of CM patients demonstrate a reduction in the tight junction proteins (ZO-1, occludin and vinculin) of brain endothelial cells, which may be the cause of leakage of BBB. Activation of the neural cells such as astrocyte and microglia were also observed. This is probably due to the direct and indirect damage caused by PRBC sequestered in the microvessels. In this study, an immortalised human brain endothelial cell line (HBEC) were used to examine the indirect effect of PRBC-HBEC contact.

Supernatants from PRBC-HBEC co-cultures were collected and applied onto fresh HBEC monolayer to test its effect on the HBEC monolayer integrity, using (1) electrical cell-substrate impedance sensing (ECIS) and (2) FITC-dextran permeability assay. Both assays demonstrated that the HBEC monolayer integrity was reduced up to 2-fold in response to PRBC-HBEC co-culture supernatant compared to control supernatants. Interestingly, analysis of the co-culture supernatant showed the induction of the ADAMTS family of proteases, ADAMTS-4. In addition, differential regulation of ADAMTS-1 and the matrix metalloproteases (MMP) family proteases, MMP-2 and MMP-9 was demonstrated. We propose that proteases are released by HBEC as a result of interaction PRBC during sequestration. This may contribute to BBB breakdown in CM.

Ursula Püntener, Steven G. Booth, V. Hugh Perry, Jessica L. Teeling

Contribution of the blood brain barrier (BBB) in neuro-immune communication following bacterial infection

CNS Inflammation Group, Centre for Biological Sciences, University of Southampton

Systemic bacterial infections lead to a powerful host immune response involving phagocytes, natural killer cells and activation of the complement cascade. This is followed by an adaptive immune response leading to immunity against the pathogen. This well-coordinated process is not only controlled by the peripheral immune system: inflammatory mediators released following bacterial infection communicate with the brain, resulting in metabolic and behavioural changes that help fight the infection. The BBB plays a critical role in this process, but changes in BBB under chronic low grade systemic infection are not well understood. We exposed mice to a systemic *S. typhimurium* infection, which led to a long lasting activation of innate immune cells of the brain, characterised by MHCII expression on the brain endothelium and a microglia hyper-responsiveness when restimulated by LPS. We also observed an increase of brain pro-inflammatory cytokines, increased influx of plasma proteins and enhanced brain T cell infiltration in *S. typhimurium* infected mice. Our study shows that systemic infection with non-neurotropic bacterial infection has long-term consequences for homeostasis in the brain, due to activation of the cerebral vasculature and priming of microglia. The prolonged and enhanced neuroinflammation in infected mice may have a detrimental effect on neuronal function.

Nichola Fletcher, Zania Stamataki and Jane A. McKeating

CD4+ T cells and inflammatory cytokines potentiate Hepatitis C virus infection of the BBB

Centre for Human Virology, University of Birmingham, Birmingham B15 2TT, UK

Hepatitis C virus (HCV) is a positive-strand RNA flavivirus that primarily infects hepatocytes within the liver. Infection induces a chronic liver injury leading to cirrhosis and hepatocellular carcinoma. However, HCV infection is also associated with extrahepatic symptoms including neurological defects. We recently demonstrated that endothelial cells of the BBB express all of the essential receptors required for HCV entry and the virus can infect and replicate in brain derived endothelial cell lines (hCMEC/D3 and HBMEC) (Fletcher et al, 2012 Gastroenterology). Extracellular HCV particles can infect brain endothelial cells, however CD4+ T cell-associated virus could infect the cells more efficiently providing an additional route of virus targeting the BBB. Moreover, inflammatory cytokines secreted from activated immune cells modulate HCV infection of brain endothelial cells. Polarization limits HCV infection of brain endothelial cells due to restricted access of viral particles to tight junction proteins which are used as viral entry receptors. Inflammatory cytokines, including TNF-α, depolarize these cells and potentiate infection. These data provide novel insights into the role of immune cells and their secreted factors in HCV infection of the brain, and highlight new pathways for HCV to hijack the inflammatory microenvironment to promote its own infection.

Dongsheng Wu[1], Camilla Cerutti[1], Miguel A. Lopez-Ramirez[1,4], Gareth Pryce[2], Josh King-Robson[2], Mark C. Hirst[1], Basil Sharrack[3], David Baker[2], David K. Male[1], Gregory J. Michael[2], Ignacio A. Romero[1]

Brain endothelial miR146a inhibits leucocyte adhesion through targeting NFAT5 to inactivate NF-κB signaling pathway

1. Department of Life, Health and Chemical Sciences, Biomedical Research Network, The Open University, Milton Keynes, MK7 6AA, UK. 2. Center for Neuroscience and Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, E1 2AT, UK. 3. Department of Neurology, Sheffield Teaching Hospitals NHS Trust, University of Sheffield, Sheffield, S5 7AU, UK

Abstract

Proinflammatory cytokine-induced activation of nuclear factor, NF-κB plays an important role in leukocyte adhesion to, and subsequent migration across, brain endothelial cells, which is crucial for the development of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Delineating endothelial intrinsic NF-κB inhibitors may help to develop new therapeutic strategies. MicroRNAs have emerged as new effectors and regulators of NF-κB. In this study, we report that microRNA-146a (miR-146a) was upregulated in microvessels of MS active lesion and the spinal cord of EAE mice at the acute stage. *In vitro*, TNFα and IFNγ treatment of human cerebral microvascular endothelial cells (hCMEC/D3) led to upregulation of miR-146a. Endothelial over-expression of miR-146a prevented cytokine-stimulated adhesion of Jurkat T cells to hCMEC/D3 cells accompanied with decreased nuclear translocation of NF-κB, an effect associated with miR-146a-mediated repression of nuclear factor of activated T cells 5 (NFAT5). siRNA mediated-knockdown of NFAT5 exhibited similar effects as miR-146a on NF-κB activities in terms of modulating expression of VCAM1 and CCL2 and leukocyte adhesion. Our study has identified miR-146a as an endogenous NF-κB negative feedback regulator in brain endothelial cells.

J. J. Connell[1,2*], G. Chatain[1], B. Cornelissen[1], K. A. Vallis[1], A. Hamilton[1,2], L. Seymour[3], D. C. Anthony[2] and N. R. Sibson[1]

Selective permeabilisation of the blood-brain barrier at sites of metastsis

[1]CRUK/MRC Gray Institute for Radiation Oncology and Biology, Department of Oncology, University of Oxford, Churchill Hospital, Oxford, UK. [2]Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, UK. [3]Department of Oncology, University of Oxford, Old Road Campus Research Building, Oxford, UK
*john.connell@oncology.ox.ac.uk

Background: Over one in five cancer patients will develop brain metastases and prognosis remains poor. Effective chemotherapeutics for primary systemic tumours have limited access to brain metastases owing to the blood-brain barrier (BBB). The aim of this study, therefore, was to develop a strategy for specifically permeabilising the BBB at sites of cerebral metastases.

Methods: BALB/c mice were injected intracardially to induce brain metastases. At day 13 after metastasis induction either tumour necrosis factor (TNF) or lymphotoxin (LT) was administered intravenously, and 2-24 hours later gadolinium-DTPA, horseradish peroxidase or ^{111}In-BnDTPA-Tz injected intravenously. BBB permeability was assessed *in vivo* using gadolinium-enhanced T_1-weighted magnetic resonance imaging and confirmed histochemically. Brain uptake of ^{111}In-BnDTPA-Tz was determined using *in vivo* SPECT/CT. Endothelial expression of TNF receptors was determined immunohistochemically in both mouse and human brain tissue containing metastases.

Results: Localised expression of TNFR1 was evident on the vascular endothelium associated with brain metastases. Administration of TNF or LT dose-dependently permeabilised the BBB to exogenous tracers selectively at sites of brain metastasis, with peak effect at 6h. Metastasis-specific uptake of ^{111}In-BnDTPA-Tz was also demonstrated following systemic cytokine administration. Human brain metastases displayed a similar TNF receptor profile compared to the mouse model, with predominantly vascular TNFR1 expression.

Conclusions: These findings describe a new approach to selectively permeabilise the BBB at sites of brain metastases, thereby enabling detection of currently invisible micrometastases and facilitating tumour-specific access of chemotherapeutic agents through sensitive detection with preclinical imaging modalities. We hypothesize that this permeabilisation works primarily though TNFR1 activation and has the potential for clinical translation.

F.E. O'Brien[1,2,3], G. Clarke[1,4], T.G. Dinan [1,4], B.T. Griffin[2], J.F. Cryan[1,3]

P-glycoprotein inhibition as a strategy to augment antidepressant delivery across the blood-brain barrier: preclinical pharmacokinetic and pharmacodynamic studies

[1]Alimentary Pharmabiotic Centre; [2]School of Pharmacy; [3]Department of Anatomy & Neuroscience; and [4]Department of Psychiatry, University College Cork, Cork, Ireland

Up to 60% of patients suffering from major depressive disorder fail to adequately respond to currently available antidepressant treatments. Mounting evidence from preclinical and pharmacogenetic studies indicates that antidepressant efflux by P-glycoprotein (P-gp) at the BBB may result in sub-therapeutic brain concentrations of certain antidepressant drugs in some patients, thereby contributing to the high prevalence of treatment resistant depression. The effect of P-gp on the pharmacokinetics and pharmacodynamics of various antidepressant drugs was investigated in the present preclinical studies.

In vitro bidirectional transport studies, using the MDCKII-MDR1 cell-line, revealed that the antidepressants imipramine and escitalopram were transported substrates of human P-gp, whereas amitriptyline, duloxetine, fluoxetine and mirtazapine were not. Microdialysis-based pharmacokinetic studies in the rat demonstrated that pharmacological inhibition of P-gp, by either verapamil or cyclosporin A (CsA), resulted in increased brain concentrations of imipramine and escitalopram, without altering their plasma pharmacokinetics. Moreover, pharmacodynamic studies revealed that inhibition of P-gp can alter behavioural responses to escitalopram.

Taken together, these findings indicate that P-gp may play a key role in limiting the brain concentrations of imipramine and escitalopram in humans. Moreover, inhibition of P-gp may represent a potentially viable strategy to augment treatment with certain antidepressants clinically. Further studies are now warranted to evaluate the safety and efficacy of this approach.

Marie N. O'Connor[1], Sussan Nourshargh[2], John Greenwood[1].

Regional heterogeneity in the endothelial glycocalyx and basement membrane of the retinal vasculature

[1]Department of Cell Biology, UCL Institute of Ophthalmology, UCL and [2]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

The blood-retinal barrier is composed of endothelial cells expressing a thick gel-like glycocalyx which projects into the lumen and surrounded abluminally by a sheath of pericytes and basement membrane. Whilst leukocyte recruitment during inflammation is enabled by changes in the expression of adhesion molecules and chemokines, the role of the glycocalyx and basement membrane in this process remain poorly characterised. We have used retinal whole-mounts and state-of-the-art imaging technology to demonstrate that the endothelial basement membrane of the retinal vasculature exhibits low expression regions (LERs) for type IV collagen and laminin, similar to those previously observed in the peripheral vasculature, where they can be permissive sites for leukocyte extravasation. We have characterised the size of these LERs and their localisation in relation to other LERs and pericytes in normal and inflamed retinas. We have also characterised the endothelial glycocalyx in normal mouse retinas using an array of lectins, and identified a heterogeneity of composition which is especially intriguing considering emerging evidence for the importance of the endothelial glycocalyx in leukocyte recruitment.

Radka Gromnicova

Small gold nanoparticles: potential carriers of therapeutics across the blood-brain barrier

The Open University, UK

The blood-brain barrier prevents up to 95 % of drugs from entering the brain which makes treatment of brain disorders challenging. The emerging field of nanomedicine can offer help in the form of nanoparticles designed to cross the blood-brain barrier and deliver a cargo. But the first step is to identify an appropriate carrier. We investigated 4 nm gold nanoparticles, which are covalently coated with glucose. These nanoparticles entered brain endothelial cells and were able to exit the cell on the basal side, moreover, they appeared to enter the cell cytosol directly rather than via vesicles. The mechanism of entry of nanoparticles appears to be based on the biophysical properties of the plasma membrane such as its fluidity. Next, we compared uptake into and across vascular endothelia from different tissues; brain endothelial uptake was the most effective. Lastly, we used our 3D in vitro co-culture model where endothelium is cultured on top of a collagen gel containing astrocytes and in this case, the nanoparticles were observed both in the endothelium and astrocytes. Therefore, this type of nanoparticle appears to have the potential to carry a therapeutic cargo across the blood-brain barrier.

Julie Tzu-Wen Wang and Khuloud T. Al-Jamal

Chemically functionalised carbon nanotubes as potential vectors for brain delivery *in vivo*

Institute of Pharmaceutical Science, King's College London, London SE1 9NH, UK
tzu-wen.wang@kcl.ac.uk

The present study aims to investigate the potential of chemically functionalised multi-walled carbon nanotubes (*f*-MWNTs) to be used as brain drug delivery vectors. *f*-MWNTs with or without a conjugation with a non-specific antibody fragment (Fab') were radio-labelled with Indium-111 prior to injection to mice. The kinetic brain uptake of *f*-MWNTs was assessed quantitatively by γ-scintigraphy in which brain samples were collected after cardiac perfusion with heparinised saline. Capillary depletion assay was further carried out to separate brain capillaries from parenchymal tissues. Higher fraction of *f*-MWNT was observed to be associated with brain capillaries. The spatial biodistribution in brain was analysed by 3D SPECT/CT imaging at various time points after intravenous injection. The results indicated the accumulation of *f*-MWNT in certain regions of the brain which was also confirmed by autoradiography of sagittal and coronal brain sections where concentration of *f*-MWNTs in regions of thalamus and mid brain was identified.

This study opens new opportunities to use *f*-MWNT as a delivery system for therapeutic or diagnostic application in the neurological field. Further studies are still needed to illustrate the uptake mechanism through the blood brain barrier and the possibility to enhance brain uptake via the active targeting approach.

Gavin Fullstone[1,2,4,] Dr J Wood[3], Professor M Holcombe[4] and Professor G Battaglia[1,2]

Modelling the Transport of Nanoparticles across the Blood---Brain Barrier

1. Department of Chemistry, University College London, London, United Kingdom 2. MRC Centre of Medical Molecular Virology, University College London, London, United Kingdom 3. Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, Yorkshire, United Kingdom 4. Department of Computer Science, University of Sheffield, Sheffield, Yorkshire, United Kingdom

The blood---brain barrier (BBB) presents a physical barrier to the exchange of almost all molecules between the brain and the blood, restricting nearly all entry to the central nervous system (CNS) To via tightly Regulated transport mechanisms. This presents A significant bottleneck in therapeutic intervention for neurological diseases, as >98% of Small molecules and ~100% of large molecules are unable to access the brain tissue. Therefore, a coordinated strategy is required for the encapsulation and specific delivery of therapeutic molecules across the BBB. Recent interest has focused on the use of nanoparticles, functionalised to target natural transport mechanisms across the BBB, for delivery to the CNS. However, enhancing the properties of nanoparticles for optimal uptake requires rigorous testing of their physical and biological interactions. Furthermore, common *in vitro,* transwell models of the BBB, frequently used in the study of trans---BBB delivery, often demonstrate wide discord to *in vivo* models. We have constructed computational models of both *in vitro* transwells and *in vivo* capillaries. These models include considerations for nanoparticle behaviour under blood flow, particle---cell interactions and subsequent transport of particles. This permits the rapid screening of different nanoparticle compositions and moreover, helps explain disparity between *in vitro* and *in vivo* data.

Poster Abstracts

Abraham S, Moss S, Greenwood J

Apelin regulates angiogenesis by modifying VEGF signalling

Cell Biology, Institute of Ophthalmology, UCL, London, United Kingdom.

Purpose: Apelin together with its cognate G-protein coupled receptor, APJ has been implicated in retinal angiogenesis and also in retinal vascular remodelling. There have been recent reports of apelin signalling being important in OIR model of angiogenesis whereas in CNV or VLDLR mouse models of pathological angiogenesis, apelin's role shown to be redundant. The aims of this study are to 1) investigate how apelin- APJ signalling regulates vascular remodelling and angiogenesis in the context of other proangiogenic factors, 2) how the signalling from different factors could crosstalk and 3) how apelin can modify VEGF mediated vascular permeability.

Methods:

In order to assess the angiogenic response to apelin under different conditions we have employed various in-vitro models including the metatarsal ex-vivo angiogenesis assay and the organotypic co-culture angiogenesis assay. The effect of treating these assays with apelin and the effect of apelin depletion in the presence and absence of other pro-angiogenic factors such as VEGF was determined. Endothelial tube formation, was determined and quantified using angiosys software. We have performed intravitreal injection of Apelin and VEGF and assessed the vascular leakage by fluorescent angiography (FA) and are performing in-vitro studies to corroborate the in-vivo studies.

Results:

Loss of apelin resulted in a significant reduction in angiogenesis in the metatarsal angiogenesis assay. In an organotypic co-culture assay where HUVECs were grown on human dermal fibroblasts, siRNA knockdown of apelin and APJ receptor showed a significant decrease in endothelial tubule formation. Exogenous addition of apelin showed an increase in vessel branching in the co-culture assay. These angiogenesis models allow us to dissect out the effect of apelin on downstream signalling from other pro-angiogenic molecules such as VEGF. Addition of VEGF significantly increases vessel branch formation (4 fold) and total tubule length in the co-cultures in comparison to the modest increase (1.4 fold) of these parameters upon apelin treatment. When we treat VEGF and apelin together, we could observe up to 5 fold increase in the vessel branch formation. These results suggest that apelin and VEGF have a synergistic effect on vessel branch formation. Preliminary results suggest that Apelin can inhibit VEGF mediated vascular permeability as measured by FA.

Conclusions:-

Angiogenic factors regulating vessel morphogenesis are tightly regulated in their expression and co-operation in signalling. In diseases such as diabetic retinopathy, age-related macular degeneration or macular telangiectasia, however, such regulations are disrupted resulting in abnormal vasculature. The above results suggest that apelin which is involved in vessel remodelling can also modify the angiogenic as well as permeability outcome of VEGF signalling and could play a role in disease pathology.

This work was supported by a grant from the Wellcome Trust

Inês Sá-Pereira1, Daniel C Anthony1, Helen Stolp[2,3]

Neurodevelopmental response to intracerebral inflammation

[1] Experimental Neuropathology, Department of Pharmacology, University of Oxford UK [2] Department of Physiology, Anatomy and Genetics, University of Oxford, UK [3] Centre for the Developing Brain, Department of Perinatal Imaging & Health, King's College London, UK
ines.tavarespintosapereira@pharm.ox.ac.uk

The distinct extracellular microenvironment of the brain is maintained by the presence of the blood-brain barrier. This barrier is functional from the very earliest stages of brain development. However, the barrier, in the developing brain, may be more sensitive to inflammation and any inflammation-induced dysfunction may contribute to neurodevelopmental disorders. We investigated the structural and functional changes that occur in the cerebrovasculature following induction of focal inflammatory lesion at different stages of brain development. Immunohistochemistry and qRT-PCR were performed at postnatal day (P) 7, P14 and P21 4h after the injection of IL1β into the striatum of C57Bl/6 mice. At each time point, the inflammatory response was different regarding adhesion molecules, cytokines, neutrophil recruitment into the brain and microglia activation. While there were marked developmental changes in the inflammatory responses, there was minimal change in the expression of transporters at the blood-brain barrier following IL1β injection. In addition, small decrease in the expression of tight junction proteins and mouse IgG staining in the brain were observed at P14 and P21, suggesting blood-brain barrier disruption. These results clearly demonstrate that susceptibility to inflammation changes throughout development, which is likely to contribute to brain injury.

Joyce Wang

Age-related change in blood-brain barrier integrity in C57BL/6 mice

The Open University

*C. Wang., M.J. Saffrey., and I.A. Romero. Dept. of Life, Health and Chemical Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA. *E-mail: joyce.wang@open.ac.uk

The blood-brain barrier (BBB) is formed by the endothelial cells of the brain microvasculature, which control the molecular traffic between the blood and brain to maintain the neural microenvironment. MicroRNAs (miRs) are endogenous non-coding small RNAs that have emerged as important regulators of gene expression. BBB leakage in cerebral cortex has been reported in normal ageing and age-related diseases in both humans and rodents (1-3). Our preliminary data and other studies suggest the deregulation of miR levels, especially miR155, in cerebral endothelial cells (CECs) may be critical in BBB dysfunction. To date information on the mechanisms underlying age-associated BBB dysfunction and the possible role of miRs in these processes, in particular in cerebral endothelial cells is lacking. The prime goal of this project is therefore to determine the role of endothelial microRNAs in modulating BBB function in ageing mice. In this study, in addition to analysis of changes in miRs in ageing endothelial cells we are also investigating whether BBB permeability is increased in ageing mice. Here we describe initial analysis of BBB integrity in C57BL/6 mice at 3, 12, 18 and 24 months of age using labelled paracellular permeability tracers (Evans blue and/or different molecular sizes of fluorescein isothiocyanate (FITC)–dextran). This work will systematically characterise age-associated changes in BBB function in mice, which may contribute to understand the mechanism of normal ageing.

ACKNOWLEDGMENTS: This work is supported by BBSRC ‘Role of microRNAs in ageing at the blood-brain barrier: integrated studies in human and mouse models’ funding: BB/J021687/1. We are grateful to the staff of the OU BRU.

Sophie Nyborg

Investigating the mechanistics of polymersome delivery across the blood-brain barrier.

University College, London

Polymersomes are nanoscaled vesicles formed via self-assembly in water. These biocompatible carriers are ideal for drug delivery due to their amphiphilic nature, allowing for encapsulation of hydrophilic or hydrophobic compounds. Previous work in our lab has showed that intravenously administered polymersomes in mice cross the blood-brain barrier (BBB), with uptake in the hippocampus and choroid plexus observed. The mechanism by which polymersomes cross the BBB was hypothesised to be via transcytosis. A transwell assay was used to investigate the kinetics of polymersome transcytosis, and showed an increase in rate of polymersome accumulation in the abluminal compartment compared to controls without cells. Furthermore, PMPC-PDPA and angiopep-POEGMA-PDPA polymersomes uptake kinetics in bEnd.3 cells and astrocytes was assessed via confocal microscopy. Intracellular uptake of angiopep-functionalised polymersomes in endothelial cells was rapid and transient, with fluorescence peaking at 30 minutes and absent after 3 hours. In contrast, astrocyte uptake was visible after 30 minutes, and remained after 6 hours. This is consistent with polymersomes entering and exiting endothelial cells via transcytosis, whereas a different intracellular trafficking route might occur in astrocytes. Future works will use a pharmacological approach of blocking receptors indicated in transcytosis, such as LRP-1.

Zerin Alimajstorovic

Investigation into the Molecular Mechanisms Underlying Idiopathic Intracranial Hypertension

The Open University

Various causative factors have been postulated as to the increasing incidence of idiopathic intracranial hypertension (IIH), however, none with any convincing evidence. Identifying biomarkers, already found to be elevated in patients with IIH, including cytokines, chemokine (C-C motif) ligand 2 (CCL2), IL-17, IL-6, IL-1β, TNF-α, leptin and hydrocortisone as to the cause of increased CSF secretion has been used to present an insight into the pathogenesis of IIH. In vivo ventriculo cisternal perfusion using both 'articial CSF only' (aCSF) and i.p injestion + aCSF, and in vitro transwell CSF secretion experiments will be used to measure volumes and rates of CSF secretion. The in vivo 'aCSF only' CSF secretion results, showed hydrocortisone (2.65 μl/min) ($p = <0.05$) to significantly increase CSF secretion rate, when compared to controls (1.90 μl/min). IL-6 (0.91 μl/min) showed a significant decrease in CSF secretion rate. The aCSF only volumes of the initial CSF within the cranium were significantly higher for leptin (289.67μl) and CCL2 (351.59μl) when compared with controls (164.17μl). Due to significant increase in initial CSF volume but decrease in overall CSF secretion for CCL2, this could indicate impairment within CSF drainage associated with this cytokine.